Research Article

Available Online at: www.ijphr.com

International Journal of Pharmaceuticals and Health care Research

ISSN: - 2306 – 6091

DEVELOPMENT OF NOVEL STEROIDAL AND NON-STEROIDAL ANTI-INFLAMMATORY AGENTS IN PRE-CLINICAL MODELS OF PSORIASIS

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Abstract

Psoriasis is a T-cell-mediated chronic inflammatory skin disease believed to be of autoimmune nature, which is characterized by circumscribed, red, thickened plaques with an overlying silver-white scale. In the present study, sensitization followed by challenge with subsequent oxazolone in acute and chronic model, observed a significant increase in ear thickness and ear weight, tissue level cytokine and circulating WBC and monocyte. In this study, we treated animal with GR agonist iso-flupredone acetate and DPC-333 either oral or systemic was found to inhibit the increase in ear thickness and weight to bring the cytokine levels down the level of prednisolone (positive control) treated animals. Also novel steroid, iso-flupredone acetate was evaluated for the first time in psoriasis pre-clinical model and demonstrated more potent effect than prednisolone. In the future complete profile might present a potential to evaluate the clinical efficacy of the steroids and provide therapeutic option in psoriasis patients.

Keywords: TNF-alpha converting enzyme, Glucocortico steroid receptor, Delays type hypersensitivity.

Introduction

Psoriasis is a polygenic, chronic relapsing autoimmune inflammatory disorder of the skin, characterized by circumscribed, red, thickened plaques with an overlying silver-white scale. It is not contagious. It occurs when the immune system sends out faulty signals that speed up the growth cycle of skin cells. It is the result of an abnormally rapid multiplication of the cells of the epidermal layer of the skin. Several different environmental factors are recognized as triggering and exacerbating psoriasis, such as infections caused by streptococci, stressful life events, alcohol consumption, smoking, diet, medications (e.g., lithium and β-blockers) and trauma. Psoriasis appears in several clinical forms such as Plaque Psoriasis, Guttate Psoriasis, Erythrodermic psoriasis, Pustular psoriasis, Nail Psoriasis, Palmar/Plantar psoriasis, Psoriatic Arthritis, Scalp Psoriasis. Psoriasis affects 2-3% of the world population[1]. The DTH-model is simple, sensitive and accurate and suitable for primary in vivo screening for anti-inflammatory activity, with a sensitising agent, e.g. oxazolone, DNFB, or FITC,[2]. Oxazolone (4-Ethoxymethylene -2-phenyl-2-oxazolin-5-one), on acute challenge in murine model exhibits primarily a Th1 response[3] that is characterized by infiltration of neutrophils and expression of TNF-α and IFN-γ[4] levels. Psoriasis

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treatment of psoriasis is based on the degree of severity. Mild and limited psoriasis treatment includes General treatments used for psoriasis A)Topical treatment: Topical Steroids (Corticosteroids), Clobetasol Propionate, Calcipotriene (Vitamin D3 derivative), Topical Retinoids (Synthetic form of vit A), Anthralin (also called Dithranol), Coal Tar, Salicylic Acid; B) Light treatment : UV-B or Ultraviolet B, PUVA (Psoralen UltraViolet-A light); C) Systemic treatment : Methotrexate, Cyclosporine, Retinoids, Alefacept , Etanercept, Adalimumab, Efalizumab, prednisolone are some examples.

The major pro-inflammatory cytokine processed by TACE is TNF-alpha which is a pleiotropic inflammatory cytokine produced by a number of cell types including macrophages, monocytes, T-cells and plays a crucial role in the pathogenesis of inflammation. Inhibition of TNF-alpha by using monoclonal antibodies, soluble TNF-alpha receptors and TACE inhibitors has been shown to suppress Th1 cytokines, activation of infiltrating leucocytes, and tissue damage and destruction[5].Some of the TACE inhibitors are currently in the clinical trials for the prevention of rheumatoid arthritis and cancer. Further, recent studies have shown that TACE inhibition could be used for inhibition of pathogenic growth factor signaling in cancer and clinical studies are underway to investigate anticancer effects of TACE inhibitor[6]. However, the exact role of the TACE inhibitor has not yet been evaluated in oxazolone induced psoriasis. Glucocorticoids are the most effective anti-inflammatory drugs available for the treatment many chronic inflammatory and immune diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases.

In the present study we propose to investigate the effects of two different classes of novel treatments, TACE inhibitor and GR agonist, in acute and chronic models of psoriasis having distinct pathophysiological conditions.

Materials and methods

Chemical and Equipment

Oxazolone (4-Ethoxymethylene-2-phenyl-2-oxazolin -5-one) – *sigma Aldrich***,** Acetone – S.d fine, Ethyl alcohol - S.d fine, PBS (Phosphate buffer saline) and 2% EDTA, Formalin, liquid paraffin, hematoxylin and eosin, ELISA kit (R&D, Minneapolis MN, USA,B.D. Bioscinces pharmingen, Bedford, USA), Micrometer (Kroeplin Langenmesstechnik, Germany, 2R20,0.0-20mm) & Homogeniser.

Oxazolone induced Psoriasis

Animals

Male balb/c mice (aged approximately 8-11 weeks) were received from Animal Research Facility of Zydus Research Centre, Cadila Healthcare Ltd. taken. The animals were kept in clean ventilated cages provided with feed and water ad libitum, in a room with controlled temperature (23° ± 3°C) and relative humidity (55 ± 10%). Protocol for use of animals for conducting this study has been reviewed and approved by Institutional Animal Ethics Committee (IAEC). IAEC protocol No. was PH-268 b (e) / 6-2K10.

Acute

Mice were sensitized with oxazolone (500 µg) dissolved in 100 µl of ethanol on the shaved abdomen. Five days after this sensitization, the mice were challenged with oxazolone (100 µg) dissolved in 20 µl of acetone on the inner and outer surface of the right ear. Control mice received the vehicle (acetone) on their right ear. Ear thickness was measured 24 h after the challenge using a micrometer (Kroeplin Langenmesstechnik, Germany, B2R20,0.0-20mm). The treatments were given 1 hour after oxazolone challenge on first and fifth day of study.

Chronic

Mice were sensitized with oxazolone, as described above. Five days later, the mice were challenged with oxazolone (100 µg) dissolved in 20 µl of acetone on the inner and outer surface of the right ear subsequent three days. Control mice received the vehicle (acetone) on their right ear. Ear thickness was measured 24 h after each challenge with oxazolone using a micrometer. (Kroeplin Langenmesstechnik, Germany, B2R20, 0.0-20mm). The treatments were given 1 hour each time after oxazolone challenge on ear.

Hematology samples

After completion of treatments, animals were bled through retro orbital route under light ether anesthesia. 300µl of the blood was collected from each animal and was mixed in 2% EDTA and was kept in ice immediately and send for the

hematological analysis for measurement of cell count such as total circulating WBC, monocyte, lymphocyte, basophil, eosinophil.

Tissue homogenization

After completion of treatments, animals were bled under light ether anesthesia and then sacrificed. Ear were collected and weighed. Ear samples were collected and kept in liquid nitrogen and they were homogenized in phosphate buffer saline (PBS) (pH-7.4) and stored at -70˚C for further assays**.**

Treatments

For Oxazolone (Acute) model

Mice were divided into six groups (n=7) as follows.

Table No. 01: No. of groups taken in acute study

Groups	Treatments	Dose
1.	Normal Control	NA
2.	Oxazolone Control	NA
3.	Iso-flupredone acetate	1% (Topical)
4	TACE inhibitor(DPC-333)	2%(Topical)
5.	TACE inhibitor(DPC-333)	(30mg/kg)p.o.
6.	GR agonist (Prednisolone)	1% (Topical)

For Oxazolone (Chronic) model

Mice were divided into six groups (n=7) as follows.

Table No. 02: No. of groups taken in chronic study

Groups	Treatments	Dose
1	Normal Control	NA
2	Oxazolone Control	NA
3	Iso-flupredone acetate	1% (Topical)
4	TACE inhibitor(DPC-333)	2%(Topical)
5	TACE inhibitor(DPC-333)	(30mg/kg)p.o.
6	GR agonist(prednisolone)	1%(topical)

The treatments (Iso-flupredone acetate and TACE inhibitor(BMS-561392,DPC-333) were dissolve in acetone vortexed for 5 min, and administered *via* topically, whereas Prednisolone was dissolve in ethyl alcohol.

Skin and Ear Histology

Skin and ear were isolated from the mice 24 h after the final oxazolone challenge respectively. The skin and ear were fixed in 10% neutral formalin, paraffinised, cut into 4-mm sections, and stained with hematoxylin and eosin (HE) for examining cell infiltration.

Enzyme-Linked Immunosorbent Assay (ELISA)

To determine the levels of cytokines *in vivo*, serum samples and ear tissue were collected 24 h after the final oxazolone challenge. IL-2, IL-1β, IL-6, Tumor necrosis factor-alpha (TNF-alpha) and Interferon-Gamma (IFN-Gamma) in serum and ear homogenate were assayed with commercially available ELISA kits (R&D, Minneapolis, MN, U.S.A.). The detection limit of each kit is 200 pg/ml for IL-2, 1000pg/ml for IL-1β, 1000 pg/ml for IL-6, 1000 pg/ml for TNF-alpha and 2000 pg/ml for IFN-gamma.

Results

Acute model

Effect of DPC-333 (either topical or systemic), Isoflupredone (topical) on ear weight in oxazolone induced psoriasis (acute)

Effect of the treatments on Ear weight

Significantly (*P<0.01)* increased in ear weight was found in Oxazolone control as compared to Normal control. Topical application of DPC 333, iso-flupredone acetate and Prednisolone treated mice showed significant *(P<0.01*) reduction in ear weight than that of oxazolone control.

Table No. 03: Effect of the treatments on ear weight

Sr. No.	Treatments	Dose	Ear weight(mg)
1	Normal control	NA	6.4 ± 0.3
2	Oxazolone control	NA	18.4 ± 1.1
3	Iso-flupredone acetate	1% (topical)	6.1 ± 0.4**
4	DPC-333	2% (topical)	9.6 ± 0.7**
5	DPC-333	30mg/kg(p.o)	18.9 ± 0.5
6	Prednisolone	1% (topical)	7.3 ± 0.5**

n=7,Values are expressed as Mean ± S.E.M.,Statistical significance was assessed as **: $p < 0.01$ When compare treated group to oxazolone treated group and #: $p < 0.01$ When oxazolone group compare with normal control group.

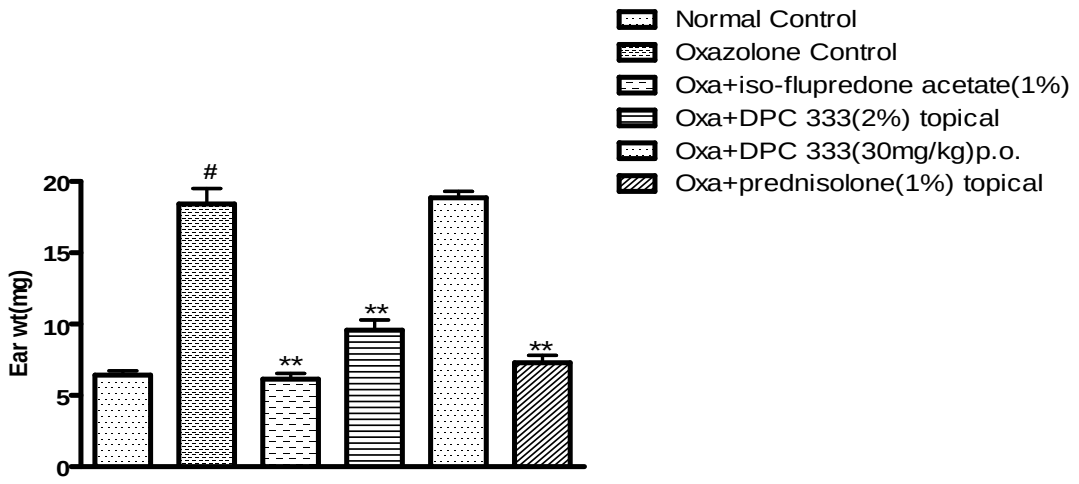

Fig. No. 01: Graph depicts effect of different treatments on ear weight after 24 hour of oxazolone challenge.

Effect of DPC-333 (either topical or systemic), Isoflupredone (topical) on ear thickness in oxazolone induced psoriasis (acute) model

Effect of the treatments on ear thickness

Significantly *(P<0.01*) Increase in ear thickness was seen in Oxazolone control as compared to Normal control.DPC 333 either topical or oral administration show significant *(P<0.01)* reduction of Ear thickness. Topical application of iso-flupredone acetate and Prednisolone treated mice showed significant *(P<0.01*) reduction in ear weight.

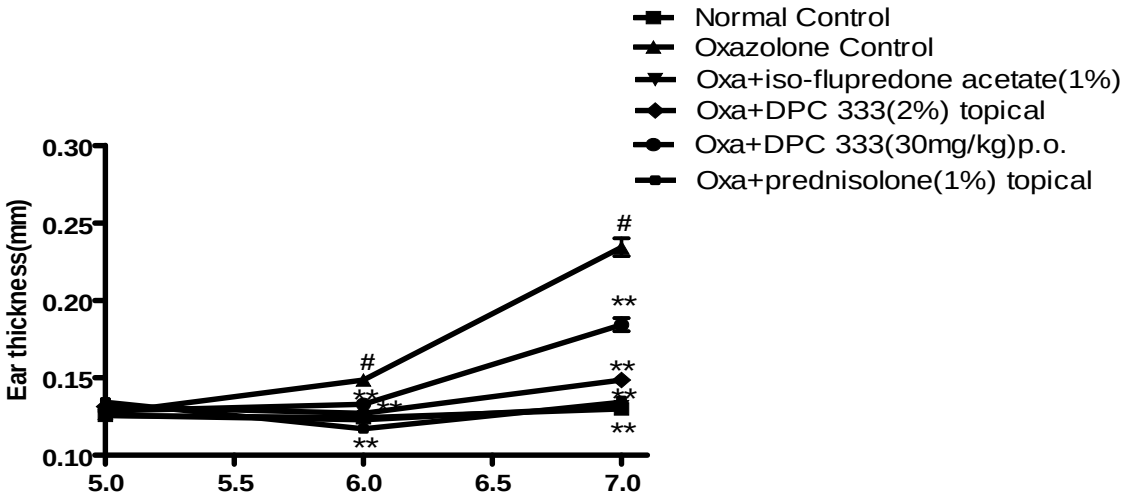

Fig. No. 02: Graph depicts effect of different treatments on ear thickness after 24 hour of second oxazolone challenge.

Table No. 04: Effect of treatments on Tumor necrosis factor-~ (TNF-~) in ear homogenate.

Sr. No.	Treatments	Dose	TNF-alpha(pg/ml)
1	Normal control	NA	66 ± 3.5
2	Oxazolone control	NA	124.9 ± 8.6
3	Iso-flupredone acetate	1% (topical)	50.1 ± 2.8**
4	DPC-333	2% (topical)	65.1 ± 4.5**
5	DPC-333	30mg/kg(p.o)	113.0 ± 11.9
6	Prednisolone	1% (topical)	66.7 ± 5.6**

n=7,Values are expressed as Mean ± S.E.M. Statistical significance was assessed as as **: $p < 0.01$ When compare treated group to oxazolone treated group and #: $p < 0.01$ oxazolone group compare with normal control group.

Effect of DPC-333 (either topical or systemic), Isoflupredone (topical) on Cytokine levels in ear homogenate in oxazolone induced psoriasis model.

Effect of treatments on Tumor necrosis factor-~ (TNF-~) in ear homogenate

Higher levels of TNF-α was found in Oxazolone control as compared to Normal control. Topical application of iso-flupredone acetate, DPC 333 and Prednisolone treatment showed significant *(P<0.01)* reduction in TNF-α levels in ear homogenate versus oxazolone group**.**

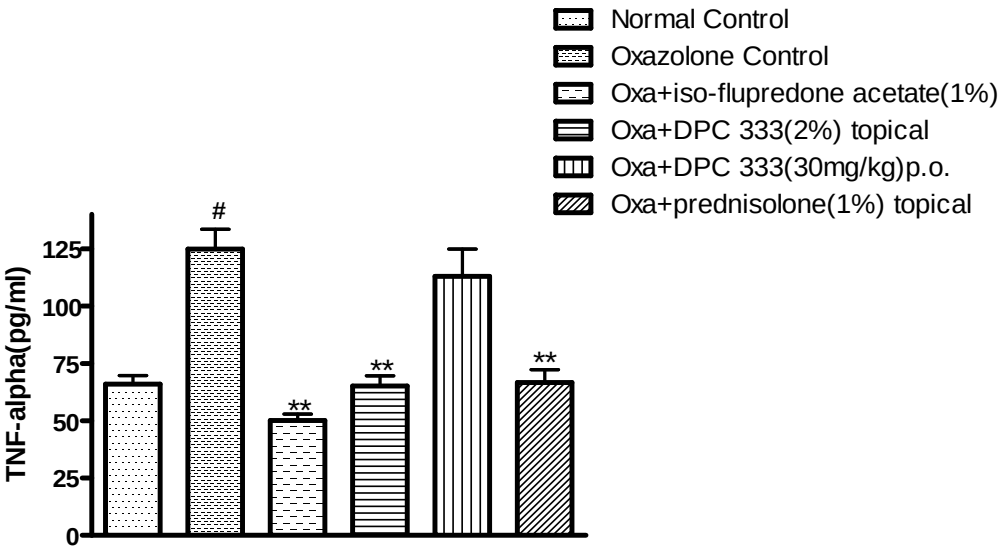

Fig. No. 03: Graph depicts effect of different treatments on TNF-α levels after second 24 hour of oxazolone challenge.

Effect of treatments on Interferon gamma (IFN-γ) in ear homogenate

Higher level of IFN-γ was found in oxazolone control as compared to Normal control. Topical application of DPC-333, iso-flupredone acetate and prednisolone treatment showed significant *(P<0.01*) reduction in IFN-γ levels than that of oxazolone treated animals.

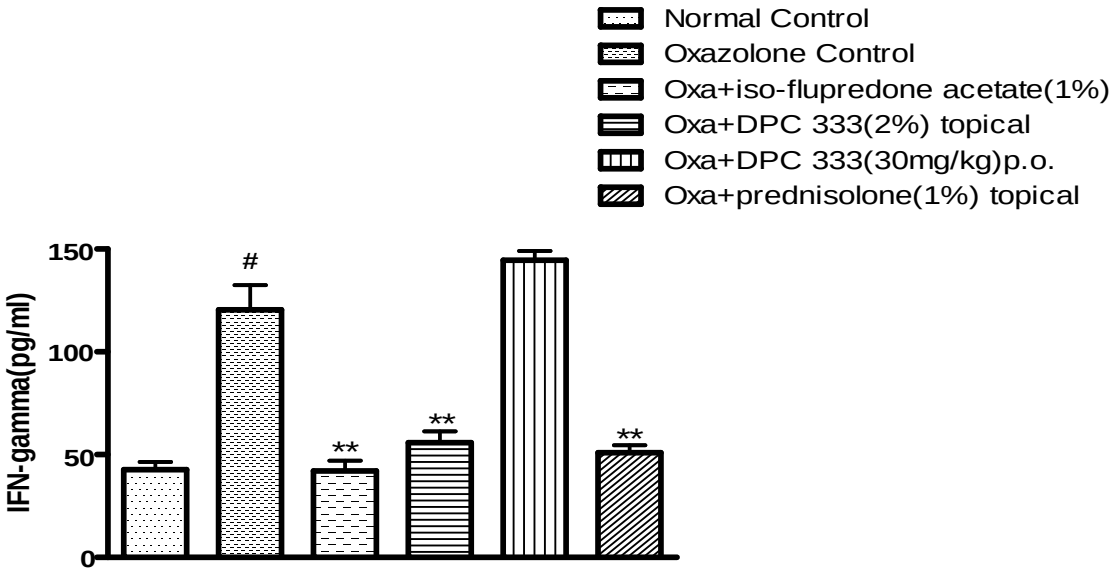

Fig. No. 04: Graph depicts effect of different treatments on IFN-γ levels after second 24 hour of oxazolone challenge.

Table No. 05: Effect of treatments on Interferon gamma (IFN-γ) in ear homogenate.

Sr. No.	Treatments	Dose	IFN-gamma(pg/ml)
1	Normal control	NA	42.7 ± 3.65
2	Oxazolone control	NA	120.4 ± 11.9
3	Iso-flupredone acetate	1% (topical)	42.1 ± 5.0**
4	DPC-333	2% (topical)	55.8 ± 5.48**
5	DPC-333	30mg/kg(p.o)	144.6 ± 4.51
6	Prednisolone	1% (topical)	50.9 ± 3.52**

n=7,Values are expressed as Mean ± S.E.M. Statistical significance was assessed as **: $p < 0.01$ When compare treated group to oxazolone treated group and #: $p < 0.01$ oxazolone group compare with normal control group

Effect of DPC-333(either topical or systemic), Isoflupredone (topical) on skin histology in Oxazolone induced psoriasis(acute)

Effect of treatments on morphology of skin tissue

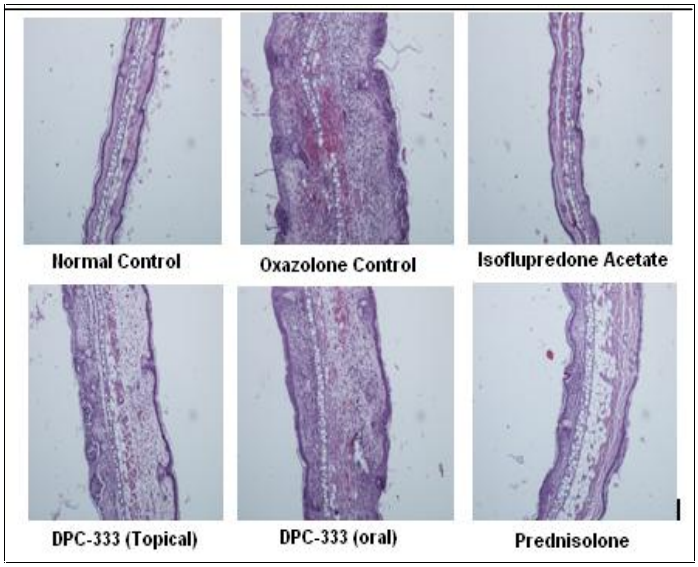

Fig. No. 05: Effect of treatments on morphology of skin tissue.

Table No. 06: Histopathological findings

Groups	Results
Normal Control	No abnormalities detected.
Oxazolone Control	Epidermal hyperplasia (Grade-3,moderate) Micro abscess with neutrophil infiltration (Grade-3, moderate) Skin erosion(Grade-2, mild) Necrosis with hemorrhages(Grade-3,moderate)
Oxa + iso-flupredone acetate(1%) topical	Micro Abscess (Grade-1,minimal) Perakeratosis(Grade-1,minimal)
Oxa + DPC-333(2%) topical	Epidermal hyperplasisa (Grade-2,mild) Micro abscess with neutrophil infiltration (Grade-2,mild) Skin erosion(Grade-1, minimal)
Oxa + DPC-333(30mg/kg)p.o.	Epidermal hyperplasisa (Grade-2,mild) Micro abscess with neutrophil infiltration (Grade-2,mild) Skin erosion(Grade-1, minimal)
Oxa + prednisolone(1%) topical	Epidermal hyperplasia (Grade-2, minimal) Micro abscess(Grade-2,minimal)

Chronic model

Effect of Iso-flupredone acetate (topical) on Ear weight and Ear thickness in oxazolone induced psoriasis.

Effect of the treatments on Ear weight:

Significant Increase ($p<0.01$) in ear weight was found in Oxazolone control as compared to Normal control. Topical application of iso-flupredone acetate and Prednisolone treated mice showed significant reduction ($p<0.01$) in ear weight than that of oxazolone control.

Table No. 07: Effect of the treatments on Ear weight

Sr. No.	Treatments	Dose	Ear weight(mg)
1	Normal control	NA	6.1 ± 0.34
2	Oxazolone control	NA	19.1 ± 0.7
3	Iso-flupredone acetate	1% (topical)	6.2 ± 0.4**
4	Prednisolone	1% (topical)	6.2 ± 0.18**

Mean ± S.E.M. (n=7). Statistical significance was assessed as **: $p < 0.01$ When treated group compare with oxazolone control group and #: $p < 0.01$ oxazolone treated animal compare to normal control group.

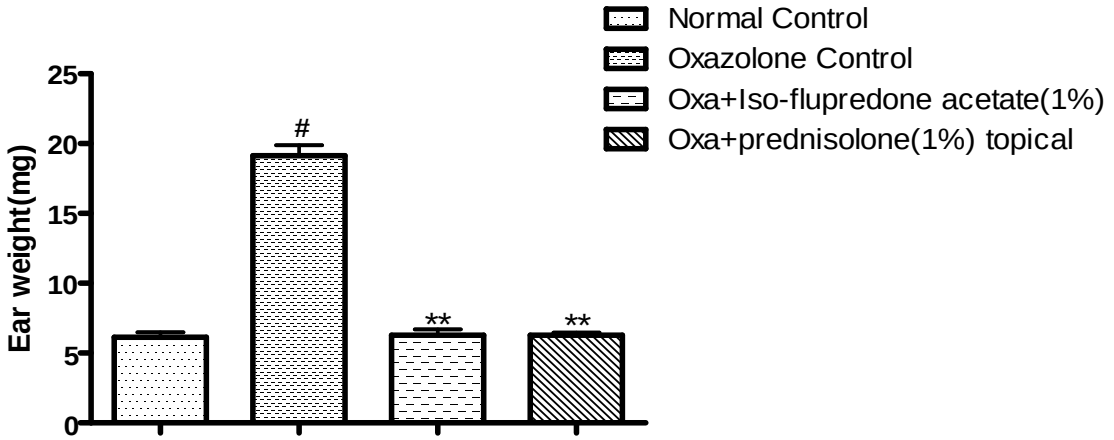

Fig. No. 06: Graph depicts effect of different treatments on ear weight after 24 hour of last Oxazolone challenge.

Effect of the treatments on Ear thickness

Increase in ear thickness was found in Oxazolone control as compared to Normal control. Topical application of iso-flupredone acetate and Prednisolone treated mice showed significant reduction in ear thickness.

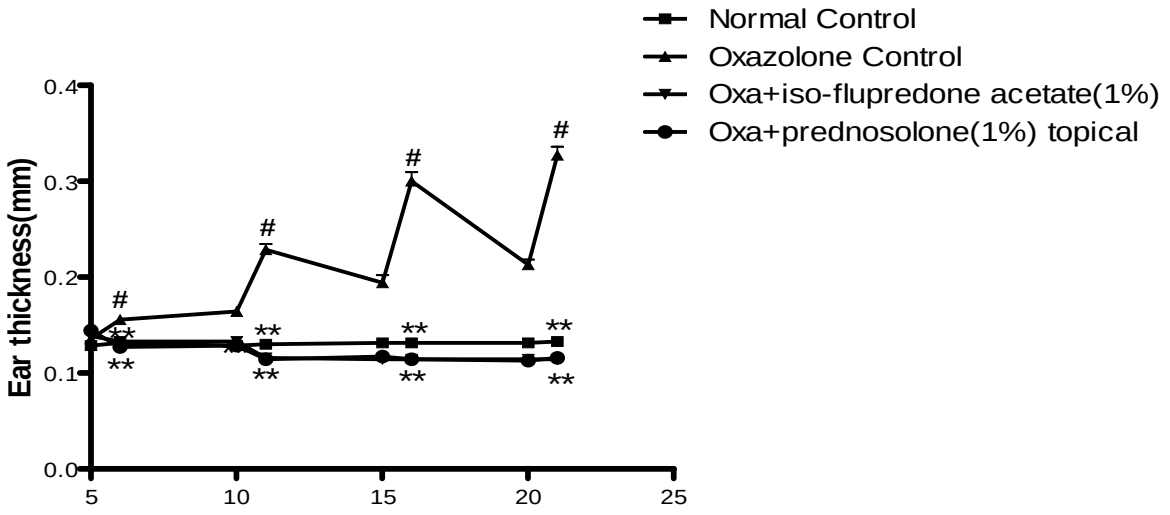

Fig. No. 07: Graph depicts effect of different treatments on ear thickness after 24 hour of last Oxazolone challenge.

N=7 Values are expressed as Mean ± S.E.M.),Statistical significance was assessed as **: p < 0.05 Vs Oxazolone control group and #: p < 0.01 vs Normal control group.

Effect of Iso-flupredone acetate (topical) on Cytokine levels in ear homogenate in oxazolone induced psoriasis

Effect of treatments on Tumor necrosis factor-~ (TNF-~) in ear homogenate

Higher level of TNF-α was found in Oxazolone control as compared to Normal control. Topical application of iso-flupredone acetate and Prednisolone treated mice showed significant reduction (p<0.01) in TNF-α levels versus oxazolone control.

Table No. 08: Effect of treatments on Tumor necrosis factor-~ (TNF-~) in ear homogenate

Sr. No.	Treatments	Dose	TNF-alpha(pg/ml)
1	Normal control	NA	66.1 ± 9.3
2	Oxazolone control	NA	134.1 ± 8.1
3	Iso-flupredone acetate	1% (topical)	43.3 ± 5.1**
4	Prednisolone	1% (topical)	45.4 ± 6.20**

Mean ± S.E.M. (n=7),Statistical significance was assessed as **: p < 0.01 vs Oxazolone control group and #: p < 0.01 vs Normal control group.

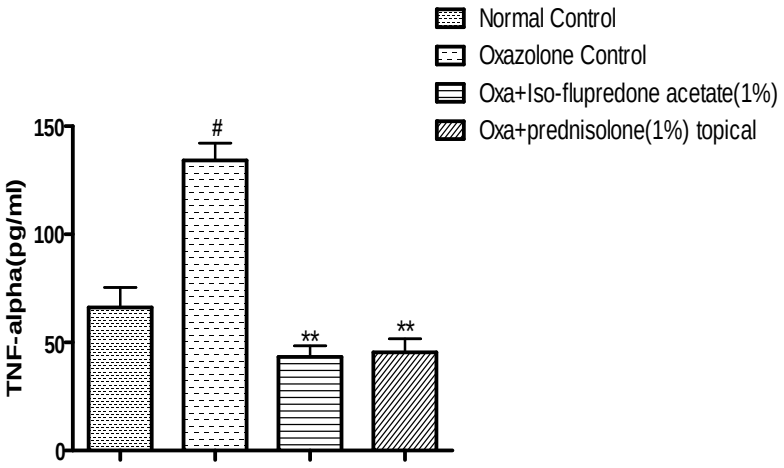

Fig. No. 0 8: Graph depicts effect of different treatments on TNF-α levels after 24 hour of last oxazolone challange.

Effect of treatments on Interferon-γ (IFN-γ) in ear homogenate

Elevated level of IFN-γ was found in Oxazolone control as compared to Normal control. Topical application of iso-flupredone acetate and Prednisolone treated mice showed significant reduction (p<0.01) in IFN-γ levels.

Table No. 09: Effect of treatments on Interferon-γ (IFN-γ) in ear homogenate

Sr. No.	Treatments	Dose	IFN-gamma(pg/ml)
1	Normal control	NA	118.8 ± 19.3
2	Oxazolone control	NA	262.3 ± 13.69
3	Iso-flupredone acetate	1% (topical)	89.7 ± 18.0**
4	Prednisolone	1% (topical)	92.5 ± 9.2**

Mean ± S.E.M. (n=7),Statistical significance was assessed as **: p < 0.01 vs Oxazolone control group and #: p < 0.01 vs Normal control group.

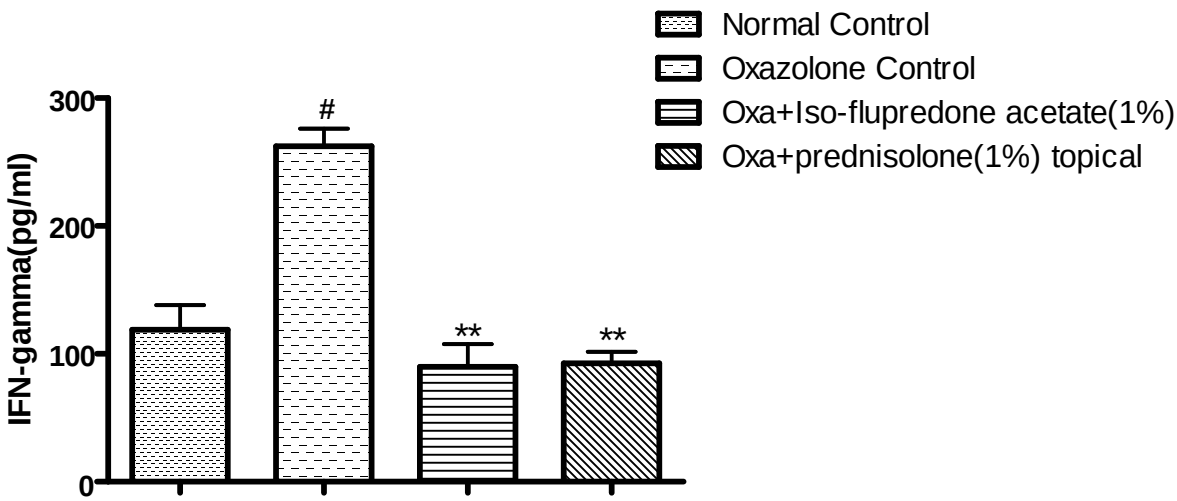

Fig. No. 09: Graph depicts effect of different treatments on IFN-γ levels after 24 hour of last oxazolone challange.

Effect of DPC-333(either topical or systemic),Isoflupredone (topical) on skin histology in Oxazolone induced psoriasis (chronic)

Effect of treatments on morphology of skin tissue

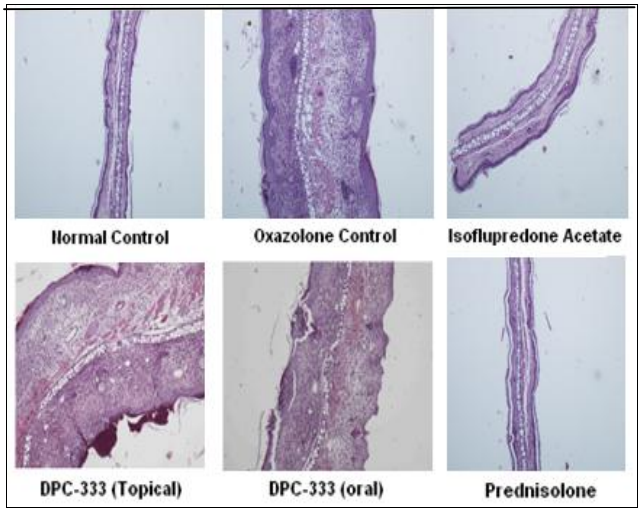

Fig. No. 10: Effect of treatments on morphology of skin tissue.

Table No. 10: Histopathological findings

Groups	Results
Normal Control	No abnormalities detected
Oxazolone Control	Epidermal hyperplasia (Grade-4,severe) Micro abscess with neutrophil infiltration (Grade-4,severe) Skin erosion(Grade-4,severe) Necrosis with hemorrhages(Grade-3,moderate)
Oxa+ iso-flupredone acetate(1%) topical	No abnormalities detected
Oxa+DPC-333(2%) topical	Epidermal hyperplasia(Grade-4, severe) Micro abscess with neutrophil infiltration (Grade-4,severe) Skin erosion(Grade-2, mild)
Oxa+DPC-333(30mg/kg)p.o.	Epidermal hyperplasia (Grade-4,severe) Micro abscess with neutrophil infiltration (Grade-4,severe) Skin erosion(Grade-2, mild)
Oxa+prednisolone(1%) topical	No abnormalities detected

Conclusion

In conclusion, our result reveals that, local application of TACE inhibitor (DPC-333) was effective in acute inflammation, not with chronic models of psoriasis, whereas oral TACE inhibitor was effective in chronic model. Also novel steroid,iso-flupredone acetate was evaluated for the first time in psoriasis pre-clinical model and demonstrated more potent effect than prednisolone.The result warrant a more detailed study of iso-flupredone acetate in other forms of psoriasis models.In the future complete profile might present a potential to evaluate the clinical efficacy of the steroids and provide therapeutic option in psoriasis patients.

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