COMPARISON OF LOCAL ANTI-INFLAMMATORY POTENCY OF CLOBETASOL, MOMETASONE AND HYDROCORTISONE IN GUINEA PIGS.

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ABSTRACT
Objectives: To compare the anti-inflammatory potency of three topical corticosteroids Clobetasol, Mometasone and Hydrocortisone by histamine induced wheal suppression test in guinea pigs. Material and Methods: Adult male guinea pigs were placed in 4 groups with 6 animals in each group. Each animal received one of the three test drugs, i.e. Hydrocortisone, Mometasone or Clobetasol and one group was kept as control. The back of the guinea pig was shaved and histamine was injected intradermally to produce a wheal and flare reaction. Dose and time required for suppression of the wheal was noted in each group and flare was graded by Visual Analogue Scale. One way analysis of variance (ANOVA) test was applied to compare the time taken for wheal suppression by the test groups, P-value 0.05 was considered significant. Results: Earliest suppression of the wheal was shown by Clobetasol group with minimal dose (0.05%), followed by Mometasone (0.1%) and Hydrocortisone with maximum dose (1%). The flare produced in clobetasol group was mild, while that in Mometasone was moderate and Hydrocortisone was severe. Conclusion: Clobetasol belonging to the most potent group showed earliest and complete suppression of wheal and flare reaction with minimal dose followed by Mometasone having intermediate potency while Hydrocortisone which was least potent did not show complete suppression of the wheal till last observation even with maximum dose. KEYWORDS: Topical corticosteroids, Clobetasol, Mometasone, Hydrocortisone.

INTRODUCTION
Corticosteroids are one of the most commonly used drugs/hormones in clinical practice for the treatment of various inflammatory diseases. Their utility ranges from various dermatological conditions to life threatening emergencies like status asthmaticus and anaphylactic shock.[1,2] Among their varied systemic uses, topically they have a immunosuppressive, anti-inflammatory, anti-proliferative and vasoconstrictive effects.[3,4] A unique aspect of dermatological pharmacology is the direct accessibility of the skin as a target organ for topical application of corticosteroids for the diagnosis and treatment of various dermatological conditions in which they can also be used in conjugation with systemic medication.[5] Topical preparations of these drugs are known to achieve high drug concentrations in superficial layers of the skin as compared to their oral administration and hence they are the most preferred route and class of drugs for treating various inflammatory dermatological conditions.[6] With the availability of so many preparations it becomes increasingly difficult for the clinician to choose the correct drug for a specific condition. Decision should be based on correct diagnosis, selection of proper class of drug along with required potency, delivery vehicle, frequency of application, duration of therapy, associated adverse effects and proper patient profiling.[7] Potency of corticosteroid can be assessed by measurement of its vasoconstricting properties and altered in the structure like halogenations, methylations etc.[8,9]

The corticosteroids have been generally classified according to their potency.[9,10] Few studies have been done to compare the effect of various topical corticosteroids. The present study was planned to compare the anti-inflammatory potency of three topical corticosteroids i.e. Clobetasol (high potency), Mometasone (intermediate potency) and Hydrocortisone (low potency) on histamine induced wheal and flare reaction in guinea pigs and to confirm whether there is a co-relation between the potency and early suppression of inflammation in dermatological conditions.
MATERIAL AND METHODS

Animals The study was carried out in adult male guinea pigs of the English Cavy species obtained from Haffkine’s Institute, Parel, Mumbai–12, weighing between 200–300 gm. Animals were kept in standard laboratory conditions, under natural day & light cycles & maintained at a humidity of 60± 5% of temperature of 25± 2°C. Animals were allowed free access to standard diet & tap water ab libitum & allowed to acclimatize for 1 week before the experiment. Permission from the Animal Ethics Committee of the Institute and approval from the Scientific Committee was obtained.

Drugs used Clobetasol propionate 0.05% (Taro Pharmaceuticals), Mometasone furoate 0.1% (Sandoz pharmaceuticals), Hydrocortisone acetate 1% (Perrigo pharmaceuticals) and 0.05 ml of histamine dihydrochloride (Sigma pharmaceuticals) 1 mg/ml (0.1% w/v).

Study Design The animals were placed in 4 groups with 6 animals in each group. Each animal received one of the three test drugs, i.e. Hydrocortisone, Mometasone or Clobetasol and one group was kept as a control group. After acclimatization, the back of the guinea pig was shaved with scissors and Gillette razor. To determine the amount of histamine required to produce a wheal of 1 cm after intradermal injection we first conducted a pilot study and found that 2 microgram of histamine was sufficient to produce a wheal of 1 cm. Hence, 2 microgram of histamine hydrochloride was injected intradermally in each group to produce a wheal and flare reaction, after which the site was wiped with filter paper to remove the excess histamine solution. The wheal area was clearly marked with a marker. After which the wheal area was subjected to immediate application of the test drugs in the three groups except for the control group. The preparations of test drugs used were:-
- Ointment Clobetasol propionate 0.05%
- Ointment Mometasone furoate 0.1%
- Ointment Hydrocortisone acetate 1%

The diameter of the wheal produced after injecting intradermal histamine (control group) and application of test drug in the remaining three groups was measured using a Vernier caliper and the flare was graded using a Visual Analogue Scale. A Vernier caliper is an instrument used for making accurate linear measurements. It is a precision instrument that can be used to measure internal and external distances extremely accurately. It consists of a high quality metal ruler with a special vernier scale attached with it which allows the ruler to be read with greater precision than would otherwise be possible. It provides a means of making measurements of distance (or length), the units on the rule portion are similar to those on an ordinary metric ruler, but the gradations on the vernier scale are slightly different. The number of vernier gradations is always one more than the number on rule for the same distance.\(^{[11]}\)

Photograph 1: Vernier Calliper

Visual Analog Scale
A Visual Analogue Scale (VAS) is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured.\(^{[12]}\) It is often used in epidemiologic and clinical research to measure the intensity or frequency of various symptoms.\(^{[13]}\) For example, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain.\(^{[12]}\) In this study we have used it to grade the inflammatory reaction depending on the hyperemia produced after injecting an inflammatory agent histamine (control group) and its suppression produced after application of the three test drugs. Here the hyperemia is graded as Grade I – Mild, Grade II- Moderate, Grade III- Severe and Grade IV- Very Severe.

<table>
<thead>
<tr>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Very severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Mild" /></td>
<td><img src="image2" alt="Moderate" /></td>
<td><img src="image3" alt="Severe" /></td>
<td><img src="image4" alt="Very Severe" /></td>
</tr>
</tbody>
</table>
The time required for complete suppression of the wheal in control group and after topical application of the drugs in the remaining three groups were noted, where Group 1 was control group, Group 2 was Hydrocortisone acetate, Group 3 was Mometasone furoate and Group 4 was Clobetasol propionate.

**Statistical Analysis**
ANOVA was used to compare the suppression of wheal diameter in all groups, to compare the wheal suppression of treatment group with control group at T15 and to compare the wheal suppression at T15 within test groups. P <0.05 was considered significant.

**RESULTS**
The effects of three topical corticosteroids were compared with a control group at different time interval (T) by measuring the reduction in diameter of wheal induced by histamine. T15 was the time interval chosen for comparing the wheal suppression of the drugs because in Clobetasol group complete suppression of the wheal occurred at T20 i.e. diameter was 0 after T15, so it would not have been possible to calculate and compare value of complete suppression of wheal with other groups after T15.

**Table 1:** Comparison of Mean ±SD of diameter of wheal suppression by test drugs with control at 5 min time interval after histamine induced wheal test

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T15</th>
<th>T20</th>
<th>T25</th>
<th>T30</th>
<th>T35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>1.23±0.20</td>
<td>1.23±0.20</td>
<td>1.22±0.17</td>
<td>1.21±0.08</td>
<td>1.20±0.05</td>
<td>1.19±0.10</td>
<td>1.14±0.04</td>
<td>1.17±0.01</td>
</tr>
<tr>
<td>Hydrocortisone 1%</td>
<td>6</td>
<td>1.23±0.20</td>
<td>1.20±01</td>
<td>1.16±0.21</td>
<td>1.12±0.16</td>
<td>0.8±0.07</td>
<td>0.5±0.02</td>
<td>0.2±002</td>
<td>0.1±01</td>
</tr>
<tr>
<td>Mometasone 0.1%</td>
<td>6</td>
<td>1.23±0.20</td>
<td>1.15±0.11</td>
<td>1.14±0.05</td>
<td>0.96±0.08</td>
<td>0.5±0.02</td>
<td>0.1±0.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clobetasol 0.05%</td>
<td>6</td>
<td>1.23±0.18</td>
<td>0.9±0.24</td>
<td>0.4±0.08</td>
<td>0.1±0.09</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 shows Mean±SD diameters of wheal produced after intradermal histamine injection (T0) where T is Time of wheal suppression. Readings of the wheal were taken from T0 to T35 (i.e. 35 minutes after intradermal histamine injection) by a Vernier caliper at an interval of 5 min. T 35 was the maximum time taken for most of the test drugs to produce wheal suppression therefore it was taken as a cut off time. The Mean±SD diameter of the wheal produced after histamine injection (T0) was 1.23±0.20 for all drugs and it decreased to 1.17±01 at T35 in control group, to 0.1±01 at T35 in Hydrocortisone group and to 0 at T30 and T25 for Mometasone group and clobetasol group respectively.

**Table 2:** Comparison of Mean ± SD of diameter of wheal suppression in control and test drugs at T0 & T15

<table>
<thead>
<tr>
<th>Group</th>
<th>T0</th>
<th>T15</th>
<th>P value</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.23±0.20</td>
<td>1.21±0.08</td>
<td>0.8247</td>
<td>Not significant</td>
</tr>
<tr>
<td>Hydrocortisone 1%</td>
<td>1.23±0.20</td>
<td>1.12±0.16</td>
<td>0.3176</td>
<td>Not significant</td>
</tr>
<tr>
<td>Mometasone 0.1%</td>
<td>1.23±0.20</td>
<td>0.96±0.08</td>
<td>&lt;0.0118</td>
<td>Significant</td>
</tr>
<tr>
<td>Clobetasol 0.05%</td>
<td>1.2±0.18</td>
<td>0.1±0.09</td>
<td>&lt;0.0001</td>
<td>Extremely significant</td>
</tr>
</tbody>
</table>

Table 2 shows comparison of Mean ± SD diameter of wheal suppression in control and test drugs at T0 & T15. Here, T15 was chosen for comparison because in Clobetasol group complete suppression of the wheal occurred at T20 i.e. diameter was 0 after T15, so it would not have been possible to calculate and compare value of complete suppression of wheal with other groups after T15. In the control group, suppression of wheal from T0 to T15 was not significant, p value being 0.8247. In Hydrocortisone group also the suppression of wheal from T0 to T15 was not significant, p value was 0.3176. Whereas, the suppression was significant in Mometasone group with a p value of <0.0118 and the suppression was extremely significant in Clobetasol group with a p value of <0.0001.

**Table 3:** Comparison of wheal suppression by test drugs with control at T15

<table>
<thead>
<tr>
<th>Control vs Hydrocortisone</th>
<th>Mean ± SD</th>
<th>P value</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.21±0.08Vs 1.12±0.16</td>
<td>0.246</td>
<td>Not significant</td>
</tr>
<tr>
<td>Control vs Mometasone</td>
<td>1.21±0.08Vs0.96±0.08</td>
<td>&lt;0.0003</td>
<td>Extremely significant</td>
</tr>
<tr>
<td>Control vs Clobetasol</td>
<td>1.21±0.08Vs 0.1±0.09</td>
<td>&lt;0.0001</td>
<td>Extremely significant</td>
</tr>
</tbody>
</table>

Table 3 shows the comparison of diameters of wheal Mean ± SD of test groups with control at T15. Here, T15 was chosen for comparison because in Clobetasol group complete suppression of the wheal occurred i.e. diameter was 0 after T15, so it would not have been possible to calculate and compare value of complete wheal.
suppression of wheal with other groups, hence T15 i.e. the time interval with least diameter was chosen. The p value obtained after comparison of Mean ± SD values with control group at T15 for Hydrocortisone group was 0.246 (not significant), Mometasone group was <0.0003 (extremely significant) and for Clobetasol group was <0.0001 (extremely significant).

Table 4: Comparison of wheal suppression of diameter at T15 amongst test drugs

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P value</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone vs Mometasone</td>
<td>1.12±0.16 Vs 0.96±0.08</td>
<td>&lt;0.0001</td>
<td>Extremely significant</td>
</tr>
<tr>
<td>Hydrocortisone vs Clobetasol</td>
<td>1.12±0.16 Vs 0.1±0.09</td>
<td>&lt;0.0001</td>
<td>Extremely significant</td>
</tr>
<tr>
<td>Mometasone vs Clobetasol</td>
<td>0.96±0.08 Vs 0.1±0.09</td>
<td>&lt;0.0001</td>
<td>Extremely significant</td>
</tr>
</tbody>
</table>

Table 4 shows comparison of Mean ± SD diameter of wheal at T15 within the test groups. The p value obtained after comparison between Hydrocortisone group and Mometasone group was <0.0001 (extremely significant) between Hydrocortisone and Clobetasol group was <0.0001 (extremely significant) and between Mometasone and Clobetasol group was <0.0001 (extremely significant).

Photograph 2: Showing comparison of the wheal and flare reaction of control with test drugs 10 minutes after application

![a. Control group](image1.png)

![b. Hydrocortisone acetate 1% group](image2.png)

![c. Mometasone furoate 0.1 %group](image3.png)

![d. Clobetasol propionate 0.05%group](image4.png)

Photograph 2 shows the wheal and flare reaction produced following intradermal injection of histamine in all the groups. These photographs were taken 10 minutes after application of topical corticosteroid ointments and were assessed by Visual Analog scale. The assessment was made at 10 min after histamine injection because according to our pilot study we found that after 10 min the wheal and flare had significantly suppressed in one test group. Hence it would not have been possible to assess the flare in that group or compare it with other
groups after the time period of 10 min. The most severe hyperaemia (Grade IV) was seen with control group (2a) followed by Hydrocortisone group (2b) which showed severe hyperaemia (Grade III) while hyperaemia was moderate (Grade II) after application of Mometasone (2c) and mild (Grade I) after application of Clobetasol (2d).

The results of the present study showed that, of the three locally applied corticosteroid ointments Clobetasol produced only mild flare and showed fastest and complete suppression of the wheal within 20 minutes with minimal dose i.e.0.05%, whereas Mometasone produced moderate flare with 0.1%, showed total suppression of the wheal within 30 minutes and Hydrocortisone produced severe flare and did not produce total suppression of the wheal even after 35 minutes of observation that too at maximum dose i.e.1%. This proves that Clobetasol is most potent and Hydrocortisone is least potent whereas Mometasone shows intermediate potency.

DISCUSSION

Topical corticosteroids are classified according to their potency, depending on their ability to produce vasoconstriction. They are ranked on a scale of I to VII.\(^{[60]}\) Wherein, Class I and Class II - highly potent, III,IV and V have medium potency while VI and VII have low potency.\(^{[11]}\) The test drugs that we evaluated were Clobetasol (high potency group), Mometasone (Medium potency group) and Hydrocortisone (low potency group). The ability of a drug to produce a response depends on the availability of the drug in its active state at the site of action. To ensure complete cure and higher effectiveness, a topical corticosteroid after application should provide higher concentration and greater reservoir effect at the local site of action. In case of topical corticosteroids; intraepidermal de-esterification is a major principle responsible for their metabolism and lowering of the on-site concentration of the drug. But with advances in science, biotransformation of topical corticosteroids in the skin can be modified to improve potency.\(^{[14]}\) More the lipophilicity of a steroid, greater is the rate of penetration and receptor binding capacity of the viable epidermis. Hence, with this increase in lipophilicity there is increase in potency.\(^{[14]}\) In high potency drugs like clobetasol, the halogenations of compounds prolongs the active state of the drug by resisting de-esterification. Most of the non-halogenated corticosteroids i.e. Mometasone furoate, hydrocortisone aconate, methylprednisolone aconate also have improved risk-benefit ratio.\(^{[15]}\) Mometasone is a non-fluorinated topical corticosteroid with a good safety and efficacy profile. It has shown to be effective in all parts of the body.\(^{[16]}\) Hydrocortisone is a naturally occurring glucocorticoid derived from adrenal cortex and its basic structure forms the basis of most corticosteroid molecules and led to development of various topical corticosteroids. As compared to its parent compound hydrocortisone, it showed higher anti-inflammatory activity on topical application.\(^{[17]}\)

In our study we compared the anti-inflammatory activity of these three topical corticosteroids by using histamine-induced wheal suppression test and visual analog scale in guinea pigs. When suppression of the wheal diameter was compared in all the three groups in Table 1, it was found that clobetasol showed earliest and maximum wheal suppression at T20, followed by Mometasone at T30; while with hydrocortisone maximum wheal suppression occurred at T35. Whereas in the control group at T35, there was still a significant amount of inflammation with very little wheal suppression. In Table 2, the Mean±SD diameter of wheal suppression in control and test groups at T0 and T15 were compared. We found that extremely significant (p value <0.0001) suppression of wheal diameter from T0 to T15 occurred in clobetasol group, while with Mometasone group the suppression of wheal diameter was significant (p value <0.0118). The wheal suppression shown by hydrocortisone group (p value 0.3176) and control group (p value 0.8247) was not significant. In Table 3, we compared the Mean±SD diameter of wheal suppression between control and test groups at T15, on comparison with hydrocortisone group the p value was 0.246 (not significant), with mometasone group the p value was 0.0003 (extremely significant) and with clobetasol group the p value was 0.0001 (extremely significant). In Table 4, we compared the Mean±SD diameter of wheal suppression at T15 in between the test groups, on comparing the Mean±SD diameter of hydrocortisone and Mometasone group, the p value was <0.0001 (extremely significant), between hydrocortisone and clobetasol group p value was <0.0001 (extremely significant), between clobetasol and Mometasone group p value was <0.0001 (extremely significant). In the visual analog scale, it was observed that the erythema in Clobetasol group was mild in nature (Grade I), whereas in Mometasone group was moderate in nature (Grade II), while with Hydrocortisone group was severe in nature (Grade III) and in control group it was very severe in nature. (Grade IV). Hence from our observations we found that Clobetasol (high potency) not only showed earliest and complete suppression of the wheal and flare, the dose at which it showed these effects were also the least amongst the other test drugs. Thus staying true to the concept that increased potency increases the penetrability and ensures faster and complete suppression of inflammation which is especially required in severe dermatological inflammatory conditions. Also, faster action ensures lesser amount of drug used which could curtail the cost along with local and systemic adverse effects.\(^{[15]}\) A study by Ainley-Walker et al also demonstrated that high potency topical corticosteroid preparations appeared to be more effective than low potent preparations.\(^{[18]}\) The present study can be compared with a study conducted by Takayama K et al in which they compared analgesic and anti-inflammatory effects of patches of 1% diclofenac sodium, 3.5 and
0.5% felbinac and 3.75% indomethacin in rats using Carrageenan-induced paw pad oedema model and brewer’s yeast-induced hyperalgesia model. In this study it was suggested that 1% diclofenac sodium 15 mg patch was atleast comparable to 2.5% and 3.5% felbinac patches and superior to 3.75% indomethacin patch in terms of analgesia and anti-inflammatory potential in the animal models tested. As in this study, 1% diclofenac patch is suggested to be a useful preparation, similarly according to our present study Cobetasol 0.05% ointment (topical preparation) can also be a useful local anti-inflammatory formulation in clinical practice, but further comparative double-blinded randomized clinical studies are suggested to demonstrate its effectiveness. In another study, topical clobetasol has been found to be significantly more effective than tacrolimus in the treatment of vulvar lichen sclerosus.

CONCLUSIONS
From the present study it can be concluded that the most potent topical Clobetasol preparation showed earliest suppression of local inflammation with minimal dose than hydrocortisone which was least potent but was having maximum dose, while Mometasone had intermediate potency and moderate dose. Hence, clinically clobetasol preparation may be preferred over other topical corticosteroids for suppression of severe local inflammatory conditions where quick response is necessary.

ACKNOWLEDGEMENTS
Authors are very thankful to the technical staff for their endless support and help in handling the laboratory animals and maintaining the laboratory.

REFERENCES