An Investigation of the Order of Applying an Emollient with a Topical Steroid in the Treatment of Atopic Dermatitis

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In 2007, the prevalence of Atopic Dermatitis had increased two to three-fold within the last three decades and affected 15-20% of young children (Buys, 2007). Current treatment includes the use of both steroid and emollient creams. Current suggestions for the order of application are contradictory. This study aims to examine the role of the order of application of treatments for Atopic Dermatitis (AD). Hairless mice (SKH-1) were induced to a mild AD flare-up using 2,4-dinitrochlorobenzene (DNCB) and treated with either 1% hydrocortisone (Maximum Strength Cortizone 10 cream) alone, 1% hydrocortisone followed by Cetaphil (emollient), or Cetaphil followed by 1% hydrocortisone. We assessed the efficacy of the treatments by measuring: body weights, area scores, severity scores, and IgE levels. For all measurements, there were no statistically significant differences observed between the treatment groups or between the treatment groups and the control untreated group. The findings may be useful in harmonizing human treatment plans in healthcare.

Introduction

Atopic Dermatitis (AD) presents with several challenging symptoms to everyday life and to caretakers. Studies have shown the growing importance of trying to find a treatment regimen that will decrease the negative effects of AD (Buys, 2007; Eichenfield et. al., 2014; & Watkins, J. 2015). Without a cure for AD, it is only possible to treat the symptoms of AD with moisturizers and medicated creams.

Lawton discusses uncertainties that patient and health professionals have including whether an emollient or topical steroid should be applied first when treating AD (Lawton, 2014). There is research to support the underlying assumption that applying an emollient improves treatment with hydrocortisone compared to hydrocortisone alone (Turpeinen, 1991).

Hydrocortisone is one of the most commonly used topical corticosteroids. Topical steroids such as hydrocortisone are used in the treatment of a flare-up or worsening in AD symptoms (Watkins, 2015) and are usually applied daily (Leung, 1998). AD is a complex disorder, making hydrocortisone's mechanism of action difficult to determine completely. Mehta et. al.'s (1998) proposed mechanism is seen in Figure 1. Mehta et. al. (1998) proposed that after the DNA binding site of receptor is exposed, there are two ways that the mechanism will branch off. This is a result of either the presence or absence of an inflammatory stimulus. Unfortunately, there are still many unknowns about the way the inflammation affects the mechanism of protein alterations, making this one possible proposal for hydrocortisone's mode of action. Emollients act on the epidermis, creating an occlusive barrier and preventing water loss from the skin. Creams are the most common delivery system for an emollient. Creams are a topical formulation known for a two-phase treatment (emulsion); two immiscible liquids, one substance in the other (Lodén, 2003). Cetaphil, according to Hon, Leung, and Barankin (2013) is a barrier cream that contains dimethicone, a water-repellent substance, that helps to protect the skin from irritants and repeated hydration.

Eichenfield et.al. (2014) defined a guideline that acute areas of AD are recommended to have once-daily application until the affected area has significantly improved or is less thick. Buys (2007) looked at clinical trials and showed that topical corticosteroids (hydrocortisone in the experiment) are effective when used up to four weeks based on previous research (Lebwohl, 1999; Maloney et. al., 2002; Sears, Bailer, & Yeadon, 1997). These clinical trials also show that in many cases symptoms may be controlled within a shorter treatment time (Buys, 2007; Lebwohl, 1999; Maloney et. al., 2002; Sears, Bailer, & Yeadon, 1997).

In the present study, we evaluated the hypothesis that the order of application of 1% hydrocortisone cream and Cetaphil (emollient) is significant to the treatment of AD in a two-week treatment period. The effects of the orders were assessed using hairless mouse model of 2,4-dinitrochlorobenzene (DNCB) – induced AD.

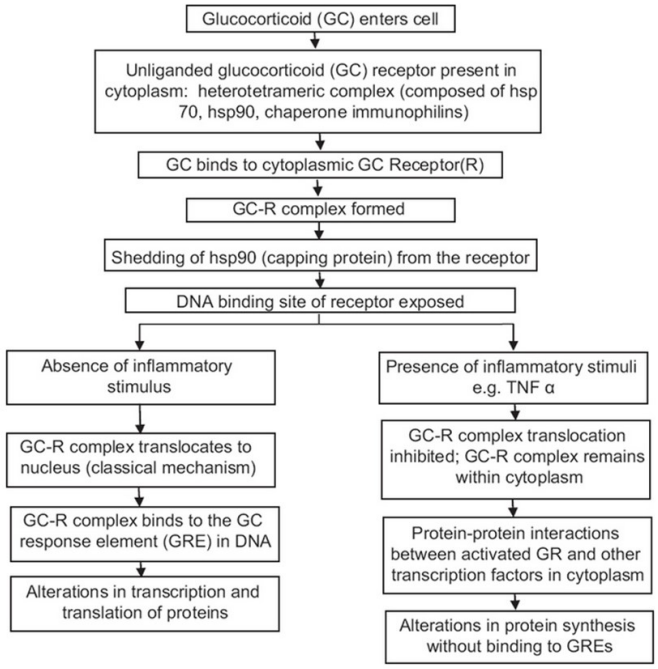

Figure 1. Proposed mechanism of glucocorticoid (hydrocortisone) action (Mehta et. al., 2016).

Methods

Animals. Three-week-old male SKH-1 Elite mice were purchased from Charles River and all the procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals: Eighth Edition (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory, 2011). Before the experiment started, the animals were acclimated for nine days. The animals were housed in a temperature and humidity controlled (22°C) room with a 12-hour light and dark cycle. The mice were also allowed free access to food and water throughout the experiment.

Grouping. Mice were randomly divided into four groups: control without treatment, hydrocortisone-treated group only, hydrocortisone applied first and after 30 minutes Cetaphil was applied, and emollient applied first and after 30 minutes hydrocortisone was applied. When the

mice were obtained, they were immediately weighed, ear punched (for identification), and were randomly selected for each cage (four mice in each cage, with one cage of five). At the end of acclimation, the cage numbers were placed into a container and drawn at random to assign the treatment/no treatment that each of the cages would receive.

Induction of AD. Induction of AD was performed using DNCB, as previously described (Kim et al., 2014), with minor modification. DNCB was mixed in a solution of 1:4 acetone and olive oil, respectively, to make a 1% solution. The solution was then placed on an adhesive gauze (clear spot Band-Aid). The Band-Aids were placed on the lower back of the mice for 24-hour periods on days 10, 12, 17, and 19 of the study (Figure 2).

Treatment. Once the AD was induced, treatment began on day 20 (Figure 2). Hydrocortisone cream (Maximum Strength Cortizone 10 cream) 0.9 grams was measured out using a scupula and scale. Hydrocortisone was applied to the back of the mice where the AD-like lesions were located. Once applied the scupula was measured again to try to reduce the amount of loss of cream and maintain an average of 0.9 grams of cream per mouse. Combined treatments used both hydrocortisone and Cetaphil (emollient used, 0.9g of the cream was measured and applied) on the backs of mice. The procedure for applying the creams to the backs were the same in all the treatments. The combined treatments had a 30-minute wait time in between the application of the first and the second cream.

Body Weight. Body weights for each of the mice were observed at every bedding change, twice weekly. Body weights were collected to observe the health of the mice and to see if there was any noticeable correlation when sensitized and treated.

Area Scores. The area scores were measured on the blood collection days (once a week following sensitization period). While the mice were anesthetized, AD-like lesion size was measured. Measurements were taken of the longest and widest dimensions of AD-like lesions.

Severity scores. Using a modified form of the EASI score as previously described by Hanifin (2001), the lesions of each mouse was scored on a scale of 0-3 for each of the following criteria: redness, scaling, and thickness. After the sensitization, the mice were scored by two members of the research team every day for the span of the experiment. The mean scores were taken from each day's scores and the standard deviations were calculated.

Blood Collection. Blood collection began immediately after sensitization on day 20 with a week between each collection (Figure 2). On blood collection days, the mice were not treated. Mice from each group were anesthetized and blood was collected via tail venipuncture. As blood pooled on the surface, it was collected in Capillary Blood Collection Tubes. The serum was collected and then frozen at 4°C until further testing.

ELISA. An IgE ELISA was performed using the serum collected. The ELISA test was conducted according to the ELISA kit instructions (BD Biosciences OptEIA Set Mouse IgE kit).

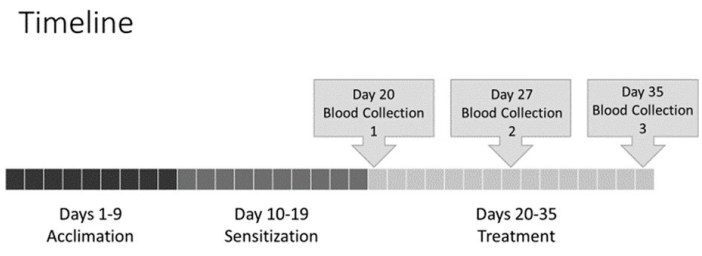

Figure 2. Timeline of the acclimation, sensitization, and treatment. Mice acclimated days 1-9 and were given free access to food and water. The mice were sensitized on days 10, 12, 17, & 19 using 1% DNCB in a vehicle of 1:4 acetone and olive oil respectively, on Band-Aids. Treatment began on day 20 and continued throughout the experiment followed by sacrifice on the 35th day.

Results

Statistical Analysis. GraphPad software (Prism, San Diego, CA, USA) was used to plot graphs. One-way and two-way analysis of variance (ANOVA) with Bonferroni's post-tests were used to perform the statistical analyses of the data.

Body Weight. As seen in Figure 3, there is a steady increase in weight during the acclimation period (the first 3 data points) this indicates the mice were growing, as to be expected with young mice (3 weeks old). There was a slower increase during the sensitization period until the second week's sensitizing that showed variation in the body weights at the end of the study. The body weights for the treatment phase climaxed on day 25 for the three treatment groups (the control dropped slightly) and continued to drop, while the control increased and leveled out for the last week of the experiment. There was no significant difference in the weights of the mice from each group at any point during the experiment indicating that there is no correlation with the significance of the order.

Area Scores. The control, hydrocortisone only, and the Cetaphil and hydrocortisone group all showed a decrease in AD-like lesion area size throughout the study (Figure 4), whereas hydrocortisone and Cetaphil showed a decrease between the first two collection days and the last day showed a slight increase in the area score. However, there were no statistically significant difference between the treatment groups in lesion area size throughout the experiment.

Severity Scores. Figure 5 shows the mean severity scores increased in all groups during the sensitization phase and continued to rise at the start of the treatment period. Each group then had severity scores return to near zero by the end of the study. There was no significant difference between the treatment groups.

IgE ELISA. As shown in Figure 6, IgE was significantly higher than control in the hydrocortisone only group on day 20 ($p<0.01$). When comparing between days in the treatment groups, neither the hydrocortisone followed by the Cetaphil or the Cetaphil followed by the hydrocortisone showed a significant reduction in IgE during the treatment period. The control group showed a significant increase in IgE between day 20 and 27 ($p<0.05$) and a significant reduction between day 27 and day 35 ($p<0.01$). The hydrocortisone only group showed no significant difference between day 20 and 27 but there was a significant decrease between day 27 and day 35 ($p<0.05$). This is likely due to such a high start value for the IgE levels for the hydrocortisone only group compared to the other groups. Looking at Figure 6 there appears to be no significance in the order of application.

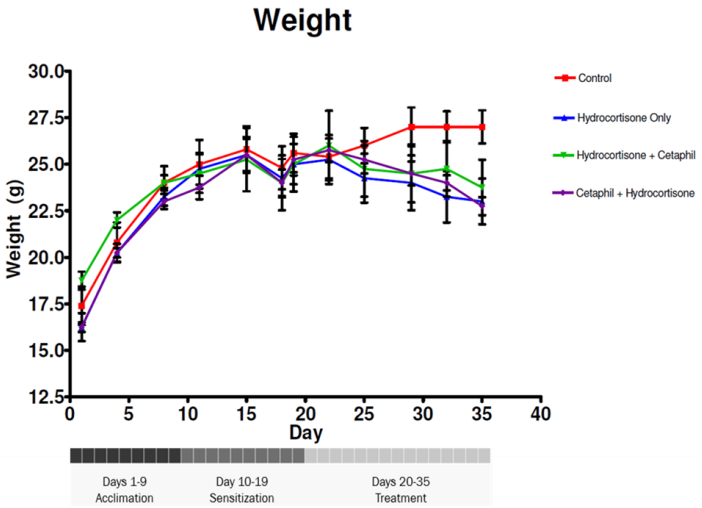

Figure 3. Representation of the mean weights of the mice in each of the treatment groups. The brackets represent the standard deviation per treatment group.

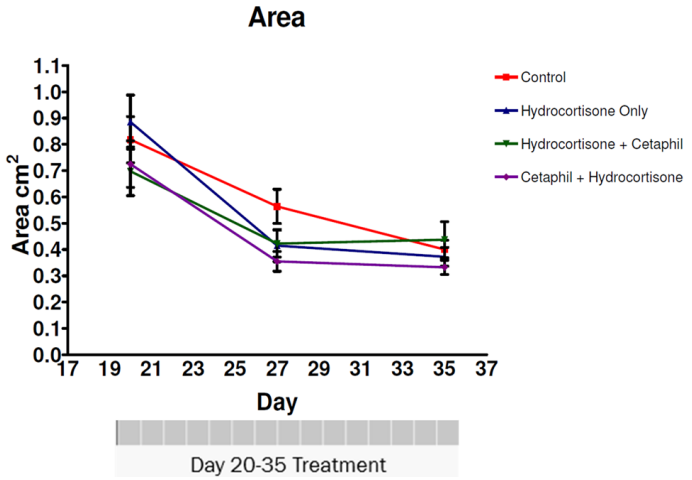

Figure 4. Mean Lesion Area - of the longest and widest AD-like lesions. The area scores decrease during the study. The control is not significantly different from the treatment regimens at any point throughout the experiment.

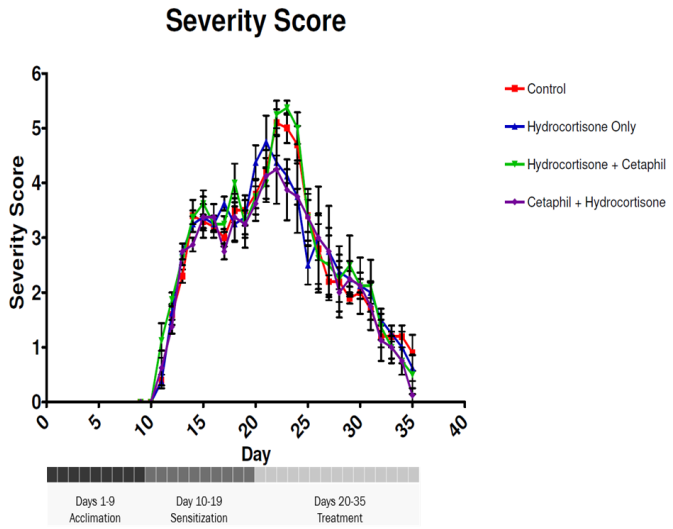

Figure 5. Mean severity scores for the treatment groups start on the 10th day throughout the study. The severity scores are a mean sum of three factors: redness, thickness, and scaling/ dryness. There is a climax of severity scores of the treatment groups at the start of the treatment phase of the experiment. The severity scores continue to decrease until the end of the study.

Discussion

There seems to be no significance to the order of application of hydrocortisone and Cetaphil when comparing weight, area, severity scores, and IgE production. The hydrocortisone-Cetaphil combination treatment may be preventing an increase in IgE (that is seen in the control group on day 27), but there was no significant decrease in IgE in these treatment groups. These results do not support the alternative hypothesis that the order of application is significant to the treatment of Atopic Dermatitis. Clinically speaking this is not far from what some doctors already thought. Smoker & Voegeli (2014) conducted a critical review looking at 27 recommendations for the order of application and the time intervals and found that there were two main treatment recommendations: topical steroid should be applied first, then wait 30 minutes and apply the emollient or apply emollient first then wait 30 minutes and apply the topical steroid. Our data concurs that the order of the application is not significant. Physicians should be able to tell the

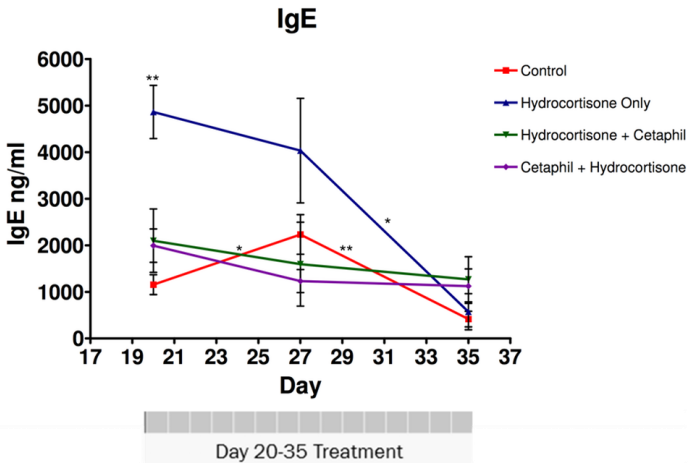

Figure 6. Above illustrates the IgE levels in the serum collected on days 20, 27, and 35. On day 11 (start of the treatment phase) there was a significant difference between hydrocortisone only and control ($p<0.01$). When comparing between days in the treatment groups, neither the hydrocortisone followed by the Cetaphil or the Cetaphil followed by the hydrocortisone showed a significant reduction in IgE during the treatment period. The control group showed a significant increase in IgE between day 20 and 27 ($p<0.05$) and a significant reduction between day 27 and day 35 ($p<0.01$). The hydrocortisone only group showed no significant difference between day 20 and 27 but there was a significant decrease between day 27 and day 35 ($p<0.05$). Looking at the comparison graph there appears to be no significance in the order of application. *$p<0.05$, **$p<0.01$.

patient that the order of the application is not significant to the treatment efficacy; as much as different articles recommending that there is at least a 30-minute absorption wait time in between the two applications (Lawton, 2014). From our data, the hydrocortisone and emollient compared to hydrocortisone shows that there is not a significant difference in the treatment regimens.

Limitations of the experiment were ways to measure area scores, blood collection, and limited sample size. The area scores were difficult to measure because some of the mice had large scabs that made it difficult to measure the area affected. The mice were small and only 100 µL of blood could safely be collected from the mice every seven days. The experiment groups only had four mice, which produces a fairly small statistical power.

Further investigation is necessary to add to the statistical power of the current study. Based on conclusions found in this experiment, future studies are needed to determine the optimum time in between the application. Also, repeat the current study with the appropriate time between applications in the combined treatment groups.

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