

Emu Oil

Anti-Inflammatory

Anti-Inflammatory & Therapeutic Properties of Emu Oil
by University of MA - Lowell, Robert Nicolosi Ph.D. (2000)

Introduction and Specific Aims:

Emu oil has been reported to have significant anti-inflammatory and therapeutic properties. It's claimed that emu oil reduces skin swelling, promotes wound healing and alleviates musculoskeletal pains, when used as an external application.

Previous studies have looked at evaluating the anti-inflammatory properties under experimental conditions, using mice as experimental animals. They found that the auricular swelling induced by 2% croton oil was significantly reduced in the high dose (5 ml) emu oil group at 6 hours, when compared to the controls. This led to the conclusion that emu oil had this beneficial anti-inflammatory property on selected sites.

The potential effect of emu oil on other tissues such as skin was not studied under in vivo conditions. It's postulated that emu oil may have similar anti-inflammatory properties in other areas of the body as well. Though it has been established that emu oil has this property, the mechanism of action is not well-understood or studied in detail.

During an inflammatory process, depending the severity and the cause, the defense mechanism of the body responds in multiple ways. The cardinal signs of inflammation are; rubor (redness), calor (increased heat), tumor (swelling), dolor (pain) and functio laesa (loss of function). Different signs manifest in different degrees and ways. Cell biology studies reveal that this complex reaction is brought forward by a group of pro-inflammatory cytokines. Inflammation is the body's reaction to invasion by an infectious agent, antigen challenge or even physical damage. This inflammatory process is actively orchestrated by the cytokines, IL-1a, TNF α and IL-6.

In this proposed study, we plan to look into the anti-inflammatory and therapeutic properties of emu oil on both auricular (ear) and cutaneous inflammation in male CD-1 mice, induced topically by application of 2% croton oil. In addition, this study is designed to measure the different cytokines levels and to assess whether there is any association between the cytokines levels and the anti-inflammatory effect. It's essential to identify and understand the mechanism of action of emu oil on inflammation.

Preliminary Studies:

To evaluate and measure the levels of different cytokines, a preliminary study was conducted in ten animals. IL-1a, TNF α and IL-6 are considered to be the predominant pro-inflammatory cytokines, in the family of interleukins that play a significant role in inducing any inflammatory response. Inner surface of right auricles (ears) of CD-1 mice were exposed to croton oil followed by application of emu oil at one hour. Animals were sacrificed at different time points: 2, 4, 7 and 9 hours post croton oil application. Two animals served as untreated controls. Exposed and unexposed ears were harvested and weighed at time of sacrifice and frozen in liquid nitrogen and saved at -85 degrees for further analysis. Blood samples were

drawn from the heart and smears made for differential counts, to evaluate whether there are any systemic reactions to this inflammatory induction. Serum was harvested from the blood and saved for cytokines levels evaluation.

Detection of mouse interleukins and other cytokines from tissue samples is done in vitro, by Enzyme Linked Immuno Sorbent Assay(ELISA) method. This is an antigen, antibody reaction and it's a quantitative determination of cytokines in mouse serum or tissue extracts (homogenized ear tissue). ELISA kit wells (Biosource, CA) coated with specific antibody are treated with sample sera or tissue extract. After a series of incubations and washing, the reaction is colorimetrically measured. The intensity of colored product is directly proportional to the concentration of the cytokine in the original specimen.

Frozen ears were digested in a tissue homogenizer containing lysis buffer. This process releases the cytokines from the cells. The extract was used to detect the levels of cytokines under consideration, measured by ELISA.

From this preliminary study and the ELISA results, it was concluded that at 5-6 hours post exposure to croton oil, the predominant cytokine responsible for this inflammatory action is IL-1 α . The emu oil mediated its' action primarily by suppressing the pro-inflammatory cytokine, IL-1 α . The other two cytokines, TNF α and IL-6 do not seem to play a significant role in elucidating this inflammatory action during this period.

Comparison of photographs taken at different time points and comparative measurements of auricular thickness and ear plug weights, strongly support the earlier findings that the anti-inflammatory properties of emu oil was greatest around 6 hours.

Materials and Methods:

Following this preliminary experiment, 34 male CD-1 mice weighing 25 to 28 grams were obtained from Charles River Laboratories (Wilmington, MA) and housed in polycarbonate cages and given commercial food and water ad libitum for 15 days before the commencement of the study. On the day of the experiment, body weights ranged from 29 to 32 grams. Animals were cared for, as per the guidelines set forth by the 'The Guide' and the Institutional Animal Care and Use Committee.

Experimental Design:

Mice were randomly assigned to three groups; Control group - untreated (n=11), olive oil control group (n=11) and emu oil treatment group (n=14). Animals were anesthetized with a combination of ketamine (100mg/ml) and xylazine (20mg/ml). Dosage: Ketamine/Xylazine 87mg/kg-13mg/kg IM. Auricular inflammation was induced by topical application of 2% croton oil to the inner surface of the right auricle. One hour later, the assigned treatment (untreated group, olive oil control group and emu oil treatment group) was carried out to each group. Dorsal skin area was clipped before the application of croton oil to the skin site. Similar treatment was carried out as for the auricles. The left ear and the left cutaneous area remained untreated and served as noninflamed controls.

Auricular thickness was measured prior to exposure of croton oil using micrometer calipers. Photographs were taken to illustrate the degree of inflammation and the treatment responses seen in each group. Animals were sacrificed at 6 hours post application of emu oil and the

thickness of the pinna measured again. Uniform size areas were punched from the treated and control sites (ears and skin) and weighed. At the time of sacrifice, blood samples were drawn from the heart. Blood smears were made for differential cell counts. Serum was harvested from the blood and saved for further cytokine analysis.

Results:

Application of 2% croton oil induced observable hyperemic inflammation and swelling in the auricles of CD-1 mice. Photographic evidence clearly illustrates this phenomenon. On the cutaneous site, no detectable hyperemia, swelling or inflammation was seen. There was edematous swelling in the right auricles and the magnitude of swelling was calculated as the weight difference between the right (inflamed) and the left (noninflamed) uniform earplugs. Pre-treatment and post-treatment thickness measurements of auricles show a significant difference in both the control and oil control groups, indicating that there was no anti-inflammatory effect in these groups. In the emu oil group, the thickness measurements (pre and post) were not significantly different. This showed that emu oil was able to reverse the inflammatory process induced by croton oil. It is also well supported by the reversal of hyperemia, compared to the control groups, as seen in the photographs (.....)

Comparison of weights of uniform ear plugs from left and right ears show that in the emu oil group there was very little difference in weight. In the control groups, the difference was very significant (statistical data)

ELISA results from serum indicate that there was no significant difference seen between the control group and the emu oil group. This indicates that the inflammatory action is more local than systemic. We expect to see significant correlation between the anti-inflammatory process and the cytokine levels from the excised ear tissues, indicating that there is suppression of the predominant pro-inflammatory cytokine IL-1a by emu oil application. (Complete results will be available in three weeks time)

Discussion:

As shown by other investigators, application of 5ul of emu oil at 5-6 hours post croton oil exposure significantly reduced the degree of inflammation in the auricles of CD-1 mice. The inflammatory process reverses itself in 24 hours, if left untreated. The natural host defense actions and mechanisms of the body bring about this reversal process. This is a complex phenomenon involving leukocyte cell migration, attachment of definitive cells to vascular endothelium and the movement of endothelial cells. Neutrophils appear early in the sites where there is acute inflammation and this is in part controlled by cytokine induction. In this study, we looked at the possible relationship and interaction between the different cytokines and the anti-inflammatory process and how emu oil mediates its' anti-inflammatory action.

Final analysis of the cytokine evaluation results will lead us to conclude the hypothesis, that the anti-inflammatory effect of emu oil is directed towards the pro-inflammatory cytokine IL-1a.

On the cutaneous site, there was no inflammation seen to the exposure to croton oil. This may be because the dorsal skin area differs histologically to the auricular site, having additional subcutaneous layers. It is possible that injecting the causative agent subcutaneously may induce a detectable inflammatory response.

