Original Research Article

Evaluation of anti-inflammatory effect of omega 3 polyunsaturated fatty acid in Guinea pigs

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A R T I C L E I N F O

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A B S T R A C T

Background: We need drugs to decrease inflammation as well as have very less side effects. Review of literature mentioned anti-inflammatory action of Omega 3 fatty acids. Hence, we selected this drug.

Aim: Our aim was to find the anti-inflammatory effect of Omega 3 fatty acid using animal model.

Study Design: The study is objective animal study where measurement of inflammation was done by observational methodology, for the present study four groups were made- 6 Guinea pigs in each group. Control guinea pigs were fed distilled water, second group standard drug indomethacin 4mg/ kg body wt, test group 1 received 1200mg/kg body wt, test group II received 2400mg/kg body wt of Omega 3 fatty acid. All drugs given orally 30 minutes before exposure to UV radiation of 30 seconds duration was given and inflammation was observed at 6, 12 and 24 hrs after UV exposure in the form of scoring.

Statistical significance was calculated using Anova method. For statistical analysis we have used SPSS system version 20.

Results: Inflammation was observed in all the four groups of Guinea pigs, there was significant anti-inflammatory effect of omega 3 fatty acid in the dose of 1200mg/kg body wt and 2400mg/kg body wt at 12 and 24 hours.

Conclusions: Omega 3 fatty acids 1200mg/kg body wt orally produced significant anti-inflammatory effect against UV radiation induced inflammation in Guinea pigs at 12 and 24 hrs. However, standard drug could produce significant anti-inflammatory effect only at 24 hrs in comparison to control group. The anti-inflammatory effects of test 1, test 2 groups and standard group are comparable.

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1. Introduction

A Roman encyclopaedist Cornelius Celsus coined the word ‘inflammation’ which comes from the Latin word inflammare (to set on fire). Inflammation is a component of the composite biological reaction of body tissues to deleterious stimuli, like pathogens, damaged cells, or irritants, and may be a defensive response involving immune cells, blood vessels, and molecular mediators.

Inflammation is important for the development of various types of diseases and disorders including autoimmune diseases neurodegenerative diseases, metabolic syndrome, cancers, and cardiovascular problems.

Inflammations are of two types: In case of acute inflammatory responses, cellular and molecular events and interactions efficiently minimize imminent injury or infection. This mitigation process contributes to restoration
of tissue homeostasis and determination of the acute inflammation. Chronic inflammation, which can be defined as a disorganized form of inflammation and organized form is called acute inflammation. The five important signs of inflammation are temperature, pain, redness, edema, and loss of function. However, unconstrained acute inflammation may become chronic, leading to a spread of chronic inflammatory diseases. It is associated with various diseases, such as atherosclerosis, osteoarthritis and rheumatoid arthritis.

This inflammatory action involves the major cells of the immune system, including neutrophils, basophils, mast cells, T-cells, B-cells, etc. Moreover, investigations of an extent of inflammatory lesions show the existence of specific leukocytes in any given lesion. The inflammatory process is controlled in such a way to make sure the appropriate leukocytes are recruited. These events are controlled by variety of extracellular mediators and regulators, including cytokines, growth factors, eicosanoids (prostaglandins, leukotrienes, etc), complement and peptides. In fact, it’s the invention of the wide ranging of those mediators over the past 20 years that has increased our appreciation of the inflammatory process although, at an equivalent time, revealing its complexity.

These extracellular events are matched by equally complex intracellular signaling control mechanisms, with the power of cells to assemble and disassemble an almost baffling arrangement of signaling pathways as they move from inactive to active roles within the inflammatory response and site.

2. Drug Review

Omega 3 fatty acid is the drug under study which is a long chain fatty acid from fish oils are known to have beneficial effects on a number of vascular risk factors in at –risk populations. The n-3 fatty acids are the long-chain polyunsaturates derived from marine oils, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the most effective variety of fatty acids. They play an important role via modification of the inflammatory activity.

These include changes in triglycerides, high-density lipoprotein cholesterol, platelet activity, endothelial and vascular function, blood pressure, cardiac function, assessment of oxidative stress, pro-and anti-inflammatory cytokines, and immune function. Further assessment of clinically important anti-inflammatory outcome or result in man are further recommended by more evaluation demonstrating benefits of n-3 fatty acids in rheumatoid arthritis, psoriasis, asthma, and inflammatory bowel disorders.

3. Methods

It is an observational study done between April 2017-June 2017 where short haired guinea pigs weighing between 280gm and 400gm were prepared for ultraviolet rays (uv) exposure in three circular areas 10mm diameter of the right flank. Each animal was exposed to uv radiation for 30 seconds. Inflammation was recorded at various time intervals (6, 12 and 24hrs) after uv exposure. The intensity of erythema was visually graded by a trained observer unaware of treatment schedules using an arbitrary scale of 0-3 as follows

1. An exposed point with no evident erythema
2. A mild reaction pale pink in colour
3. A moderate response between 1 and 3
4. An intense reaction deep reddish pink in color

A maximum score for a 3 point exposure in a single animal was 9

Each group contains six Guinea pigs.

For the study the animals were fasted overnight and the drug treatment was administered orally through animal feeding tube half an hour before the ultra violet ray exposure.

Group 1- control
Group 2- standard indomethacin 4mg/kgbody wt
Group 3- test group 1 1200mg/kgbodywt omega 3fatty acids
Group 4-test group 2 2400mg/kgbodywt omega3fatty acids

Erythema (redness) is the earliest sign of inflammation, not yet followed by plasma exudation and edema. Guinea pigs are the routinely used animals to study the anti-inflammatory activity of drugs in this model. In Albino guinea pigs, the pure erythema reaction appears 2 hrs after exposure of the depilated skin to ultraviolet irradiation. Guinea pigs are pretreated with the test drug half an hour before UV submission from a UV lamp that emits radiation in the wavelength of 180-200nm. The model can be used as a pure measure of the vasodilatory phase in the inflammatory reaction, however the test suffers from drawback that shaving of skin is required before administration of irritant. The test is based on the skin thickness and the strength of erythema. It is tough to quantify the erythema and requires an adept investigator.

3.1. Exclusion criteria

Exclude guinea pigs of dark color.

3.2. Inclusion criteria

Guinea pigs of any gender.
Table 1: Inflammation score in control, standard, test 1 and test 2 groups of Guinea pigs at 6 hours, 12 hours and 24 hours (mean+SD/SE)

| Guinea-pig_6 Hrs       | N  | Mean | Std. Deviation | Std. Error |
|------------------------|----|------|----------------|------------|
| Control                | 6  | 2.50 | .548           | .224       |
| Standard               | 6  | 2.67 | .516           | .211       |
| Test 1                 | 6  | 2.50 | .548           | .224       |
| Total                  | 24 | 2.54 | .509           | .104       |
| Control                | 6  | 2.50 | .548           | .224       |
| Standard               | 6  | 1.67 | .516           | .211       |
| Test 1                 | 6  | 1.50 | .548           | .224       |
| Total                  | 24 | 1.79 | .658           | .134       |
| Control                | 6  | 2.33 | .816           | .333       |
| Standard               | 6  | .33  | .516           | .211       |
| Test 1                 | 6  | .67  | .516           | .211       |
| Test 2                 | 6  | .50  | .548           | .224       |
| Total                  | 24 | .96  | .999           | .204       |

4. Results

One of the laboratory models of inflammation developed to test the anti-inflammatory properties of drugs is the ultraviolet induced erythema of Guinea pig skin. In control group inflammation was observed at six hours which gradually decreased at 24 hrs. In comparison to control, there was significant reduction in inflammatory score in omega 3 fatty acid in the dose of 1200mg/kg body wt and 2400mg/kg body wt at 12 and 24 hours suggesting anti-inflammatory effect of 1200mg/kg body wt and 2400mg/kg body wt of Omega 3 fatty acid. Standard drug i.e. Indomethacin 4mg/kgbody wt also showed significant decrease in the inflammatory score at 24 hrs in comparison to control group suggesting standard drug 4mg/kgbody also produced anti-inflammatory effect in the present experimental model.

As there is no significant difference between test 1 and test 2 and standard groups either at 12 hrs or 24 hrs, the anti-inflammatory effects of test 1, test 2 and standard are comparable (Tables 1 and 2). However, dose of Indomethacin was less (4mg /kg) than test 1 (1200mg/kg Omega 3 fatty acid or test 2 (2400mg/kgbodywt Omega 3 fatty acid).

Marc-Olivier Trépanier, Kathynyn et al. also revealed in a review article – neuro anti inflammatory action of Omega 3 fatty acid in experimental animals however, it is not clear by which process omega-3 polyunsaturated fatty acids exercise their outcome. Upcoming research should be done to develop and evaluate its effect.

Miller and Yamaguchi showed Guinea pig epidermis result in assumed anti-inflammatory metabolites from fish oil polyunsaturated fatty acids.

Ziboh and Cho showed that dietary supplementation of fish oil leads to limited release of cutaneous anti-inflammatory and antiproliferative metabolites may serve as less toxic in vivo monotherapies or as adjuncts to standard therapeutic regimens for the management of inflammatory skin disorders.

However, human research requiring patients who are critically ill and in ICU showed Parenteral nutrition including n-3 PUFAs revealed to preserve immune activity better than standard total parenteral nutrition and manifest to partly prevent some aspects of the inflammatory response. There may be some objective advantage from these effects. n-3 PUFAs are a component of enteral formulae that have been examined in a number of clinical trials.

Further clinical studies are to be conducted to find the safety of Omega 3 fatty acids in treatment of inflammatory diseases such as rheumatoid arthritis, as Indomethacin is known to produce side effects like peptic ulceration on prolonged usage.

5. Discussion

Omega 3 fatty acid is a polyunsaturated fatty acid is effective in treating many diseases which includes Rheumatoid arthritis, Crohn disease, Ulcerative colitis, Lupus, Type 1 and Type 2 Diabetes, childhood asthma, Allergic disease, Psoriasis, Atherosclerosis. One of the reasons for this finding is anti-inflammatory action of Omega 3 fatty acid.

In our present study both the Omega 3 fatty acid and Indomethacin showed significant anti-inflammatory effect against the inflammation produced by UV rays in Guinea pigs.

As there is no significant difference between test 1 and test 2 and standard groups either at 12 hrs or 24 hrs, the anti-inflammatory effects of test 1, test 2 and standard are comparable (Tables 1 and 2). However, dose of Indomethacin was less (4mg /kg) than test 1 (1200mg/kg Omega 3 fatty acid or test 2 (2400mg/kgbodywt Omega 3 fatty acid).
Table 2: Statistical significance of inflammation score in control, standard, test 1 and test 2 groups of Guinea pigs at 6 hours, 12 hours and 24 hours (Anova Test)(Tukey HSD)

| Dependent Variable | (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | Sig. |
|-------------------|------------|------------|-----------------------|------------|------|
| Guinea-pig_6 Hrs  | Control    | Standard   | -.167                 | .312       | .950 |
|                   | Test 1     | Standard   | .000                  | .312       | 1.000|
|                   | Test 2     | Control    | .167                  | .312       | .950 |
|                   | Standard   | Test 1     | .167                  | .312       | .950 |
|                   | Test 2     | Control    | .000                  | .312       | 1.000|
|                   | Test 2     | Control    | .000                  | .312       | 1.000|
|                   | Standard   | Test 1     | .000                  | .312       | 1.000|
|                   | Test 2     | Control    | .000                  | .312       | 1.000|
|                   | Test 2     | Control    | .000                  | .312       | 1.000|
| Guinea-pig_12 Hrs | Control    | Standard   | 2.000                 | .354       | .000 |
|                   | Test 1     | Standard   | 1.667                 | .354       | .000 |
|                   | Test 2     | Standard   | 1.833                 | .354       | .000 |
|                   | Control    | Test 1     | 2.000                 | .354       | .000 |
|                   | Test 2     | Standard   | .333                  | .354       | .782 |
|                   | Control    | Test 2     | .167                  | .354       | .964 |
|                   | Test 2     | Standard   | .333                  | .354       | .782 |
|                   | Control    | Test 2     | .167                  | .354       | .964 |

6. Conclusion

As there is no significant difference between test 1 and test 2 and standard groups either at 12 hrs or 24 hrs, the anti-inflammatory effects of test 1, test 2 and standard are comparable (Tables 1 and 2). In the present study, pretreatment with Omega 3 fatty acids(1200and 2400mg/kgbodywt) (test 1 and test 2), standard drug Indomethacin (4mg /kg body wt) orally produced significant anti-inflammatory effect in the UV rays induced inflammation in Guinea pigs.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

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