

Clinical applications of Trioxolane derivatives

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Introduction and Product Description

Several laboratory, anecdotal and clinical studies have been performed using the patented derivatives of 1,2,4-Trioxolane during a period spanning over 20 years. Observations obtained demonstrate the uniqueness of this product. The product, when properly formulated can exhibit properties ranging from being a biological response modifier, antirheumatic, immunomodulator, anti-inflammatory, antiviral, and antimicrobial. These properties become handy in evaluating the product's potential in the clinical management of various conditions such as infections and immune disorders [1].

Geraniol trioxolane, a trioxolane derivative of a terpene, has been assessed on the basis of its direct biological activity against certain target organisms *in vitro*. *In Vivo* assays have similarly been undertaken to assess the product's toxicity, safety, and in certain cases, efficacy. The studies have been conducted using the product manufactured using a synthesis protocol, and synthesized strictly in accordance with international guidelines and specifications, especially those issued by United States Food and Drug Administration, United States Pharmacopoeia and British Pharmacopoeia. The preparatory process met all requirements for the Code of Good Manufacturing Practices (cGMP). In addition, the trioxolane derivative of *cis* 3-hexene-1-ol, a non-terpene, was synthesized and compared with geraniol trioxolane for the purposes of assessing its comparative activity.

Both geraniol Trioxolane and the trioxolane derivative of *cis* 3-hexene-1-ol are colorless liquids. Geraniol trioxolane is more viscous and less stable than the trioxolane derivative of *cis* 3-hexene-1-ol.

After several trials, the more user-friendly and stable version is a patented product that contains methyl-5-octyl-1,2,4-trioxolane-3-(8'-octanoate), a derivative of 1,2,4-trioxolane,

as the active ingredient. This is a formulation manufactured and conveniently dissolved in Squalane (2,6,10,15,19,23-hexamethyltetracosane), as a carrier, using liquid preparation containing 200mg/ml of 1,2,4-trioxolane. Studies so far conducted both in Mexico and Kenya have revealed that various formulations of this product have a place in the clinical management of a variety of conditions. Clinical trials have been done to assess the usefulness of this product in the clinical management of HIV/AIDS, rheumatoid arthritis and also in the improvement of blood circulation. No adverse effects were observed in all the studies so far undertaken. Because of its wide window of safety, two products have been developed and have received registration in Kenya: these are branded *Alphamir* for HIV/AIDS and *Arthromir* for rheumatoid arthritis. Except for the initial experimental studies and anecdotal observations, most of the clinical trials have been undertaken at the Kenya Medical Research Institute (KEMRI).

HIV/AIDS

Recent studies were undertaken at the Kenya Medical Research Institute (KEMRI), involving an open label, randomized dose ranging clinical trial for efficacy, safety and tolerability of the product in asymptomatic HIV-1 individuals with CD4 counts of 100-500 cells/ μ l of whole blood.

In this study, 48 patients with proven HIV-1 status were recruited. The study consisted of three arms at buccal (oropharyngeal) dose levels of 100mg, 200mg, and 400mg daily for a period of 4 months. The patients' CD4 counts were divided into low and medium, 100-250 cells/ μ l and 251-500 cells/ μ l of whole blood respectively before random allocation to the 3-treatment dose levels. Half of the patients at each dose level were between 100-250 cells/ μ l and the

other half between 251-500 cells/ μ l of blood. Efficacy parameters were changes in the CD4, CD4/CD8 ratios and viral loads. Toxicity was assessed by monitoring liver, renal and bone marrow functions. An extra 20 patients were recruited on compassionate basis in an expanded study and studied under the same protocol.

Follow-up was done every two weeks. At each follow-up all toxicity and efficacy assessments were done and the viral loads were done every month. At the end of the study period, a total of 48 individuals had been recruited into the formal study. The first and second follow up had been done in all of them. Thirty-seven have had the third follow up while sixteen had the fourth follow up. During the period, there have been four dropouts. One was due to relocation of his work place following posting by his employer; another was due to the frequent travels in and out of the country that could not offer sufficient time for regular follow-up and visits. The reasons for the dropout of the other two were not related to use of the drug.

Before data analysis was done, all the study patients, except the drop-outs, had completed the scheduled follow-ups. Those with previous weight loss had gained an average of 1.2kg (2.6lb) by the fourth week and this rate of weight gain was maintained during the period of study or until it reached the expected weight. At the end of the follow-up period in all patients, the results indicated that the maximum reduction of log -0.3 or more in viral load was achieved in those patients on 200mg daily dose. Those on 100mg registered log -0.19 while those on 400mg registered log -0.13.

The CD4 levels have shown appreciable increase over the base line in those on daily doses of 200mg. The levels reached a plateau during the third month of treatment at an average of 30% increase over the baseline. The increase was significant. More than 70% of patients had an increase of more than 20% of their CD4 counts over the baseline while only 20% of those on 100mg daily dose had a similar increase. Less than 20% of those on 400mg had more than 20% increases in their CD4 counts. In summary, those patients on 200mg daily dose did better in terms their CD4 counts than those on either 100mg or 400mg daily dose. Observation on whether the high levels of CD4 can be sustained. Due to

constraints in resources, observations on this parameter were done in selected, but random samples. CD4 levels were have been sustained in samples tested. Further studies on this product are underway.

Rheumatoid Arthritis

This was an open label study to determine efficacy and safety of the product (Arthromir) in the management of patients with rheumatoid arthritis (RA). The study was conducted under and in accordance with approved protocols. In the expanded study, 50 patients with a clinical diagnosis of rheumatoid arthritis were enrolled after fulfilling the prescribed inclusion criteria. Trioxolane at 200mg given in four divided doses daily was given sublingually for 12 weeks. Efficacy and safety was assessed as defined in the protocol. The patients were followed up for a total of two months after completion of therapy. A similar number of patients were enrolled purely on compassionate basis, but outside the approved study, and followed up together with those on the study.

This approved study involved a two-week hospitalization. In this regard, the choice of the dates for the patients to check in was dependent largely on the individual patient's work schedules or programs.

The patients completed three-month follow-up and there has never been any recurrence of the clinical disease during the period under study. In all patients, there has been consistent response: alleviation of pain within one week of hospitalization and commencement of therapy and sharp reduction of inflammation at the affected areas.

On admission, the major majority of patients presented themselves with chronic active painful joints associated with swellings in the joints. The majority of them were unable to move the joints and edema cleared by week 1 after commencement of medication. Pain had subsided and they were able to move the joints. Pain was virtually gone by week 4. So far, subsequent follow-ups have shown that they were able to sustain pain free status, except one case whose pain recurred and required additional round of medication. After the second round of medication, the patient recovered and has since remained symptom free.

The preliminary data obtained so far have strongly suggested that Arthromir is a product that has great potential in the clinical management of rheumatoid arthritis. Its mechanism of action is novel and provides an insight into the understanding of the pathology associated with the disease. On the basis of the properties of Arthromir, there are new pieces of information which strongly suggest that the pathogenesis of arthritis needs to be looked at afresh.

Micro-capillary Circulation

This short pilot and anecdotal study was done in the United States and Kenya. Wet freshly drawn whole blood from clinically normal individuals exhibit a general cellular stickiness with massive red cell agglutination, irregular cell size and shape, and extraneous intracellular debris that includes pleomorphic granules, especially in the extremities. However, when the product is administered as prescribed, and blood drawn in about 20 minutes and examined, marked and obvious changes were seen. Within 20 minutes, the red cells had plumped up and showed more homogenous size and shape. They had "lost" the previously seen "stickiness" and now appeared less viscous and "teflonized" and moved more freely without any evidence of agglutination. Capillary perfusion showed marked improvement.

The undiluted freshly drawn fingertip blood was found to be sticking together in a rosette manner. It was, however, too close to each other to observe any changes. This necessitated the dilution of blood by 30 percent with normal saline. This dilution factor was applied in all pre- and post-treatment samples.

We have overcome the problem of dilution. The pre-treatment samples revealed that most of the cells (more than 90 percent) were sticky and floating together in a chain form. After 15 minutes following drug administration, the stickiness had reduced to 60 percent and little debris was seen floating. At 30 minutes, the stickiness had reduced to less than 40 percent and the debris eliminated. It is believed that scavenger cells had eliminated the floating debris that had been released following the de-clumping of red cells. Maximum teflonization of cells was achieved at around 30 minutes post-treatment and this condition was maintained for two hours, the longest time the observation was made.

We are currently assessing the level of activity of phagocytic cells by looking at the levels of peroxidase activity at different time intervals, and the possibility of nitric oxide involvement. Further studies notwithstanding, the present observations strongly places the product as a very good candidate in the prevention and management of stroke and heart attack, and in the prevention of debilitating complications associated with diabetes. Studies on this front are being contemplated.

1,2,4-Trioxolane Derivative as a Drug

From the foregoing, the studies on trioxolane derivatives have led to the development of two drugs which have received registration in Kenya for use in the clinical management of HIV/AIDS and rheumatoid arthritis. These drugs bear the trade names Alphamir (for HIV/AIDS) and Arthromir (for rheumatoid arthritis). Because of its properties, the trioxolane derivative has been described as a biological response modifier, antirheumatic, immunomodulator, anti-inflammatory, antiviral, and antimicrobial.

Composition and presentation

The drugs contain methyl-5-octyl-1,2,4-trioxolane-3-(8'-octanoate), a derivative of 1,2,4-trioxolane, as the active ingredient. This is a formulation manufactured and conveniently dissolved in Squalane (2,6,10,15,19,23-Hexamethyltetracosane), as a carrier, using liquid preparation containing 200mg/ml of 1,2,4-trioxolane.

Indications

Arthromir is used in the treatment of acute and chronic rheumatoid arthritis, osteoarthritis, inflammatory polyarthritis and other forms of arthropathies; systemic lupus erythematosus; and is also used in promoting micro-capillary blood circulation. *Alphamir* is used in the treatment of HIV/AIDS.

Dosage and administration

Arthromir is for both oropharyngeal and topical use depending on the condition for which it is applied. *Alphamir* is primarily for oropharyngeal use.

For oropharyngeal use, the drug should be administered as a buccal (sublingual) dose of 50mg contained in 250ul (or 10 drops containing a total of 50mg of 1,2,4-trioxolane), four times a day. Retain the drug within the

oropharyngeal area for at least five minutes after which it may be swallowed. Avoid taking anything else by mouth for at least 30 minutes following administration. Because one of the principal modes of action of Arthromir is through receptor activation at the oropharyngeal area, the dosage is not directly related to body weight but, rather, to surface area on which the drug is acting. Hence, the adult dose is similar to that of children above the age of five years.

In cases of topical manifestations as well as systemic conditions, it is possible to use both the topical preparation in the affected areas and the oropharyngeal preparation simultaneously, without inducing toxicity or overdose, and without loss of efficacy. The drug is well tolerated.

In cases of localized topical manifestations or swellings, it is possible to apply the topical preparation on the affected areas and use oropharyngeal preparation simultaneously without inducing toxicity or overdose, and without loss of efficacy.

Contraindications and special precautions

There are no known hypersensitivity reactions to the active substance or to any of the excipients. Unlike other non-steroidal anti-inflammatory drugs (NSAIDs), both drugs are safe for use in patients with allergies. In case itching or any other undesirable effect occurs, drug may be withdrawn temporarily (for about 2 days) and thereafter, resumption of treatment can begin.

Overdose

The window of safety for Arthromir is wide; hence, there is little likelihood of the drug inadvertently causing recognized toxicity due to overdose. However, caution should be taken (by taking the recommended dose) in order to limit loss of efficacy that may occur as a result of higher doses than recommended.

Pharmacodynamics and pharmacokinetics

1,2,4-Trioxolane is an endoperoxide derivative of an unsaturated fatty acid. It is metabolized *in vivo* by cleavage of the endoperoxide ring followed by beta-oxidation of the resulting short chain aliphatic molecules. The molecule is sufficiently lipophilic to be able to cross cell membranes. But due to its ready inactivation in tissues, its site of action is near the site of administration, i.e. the oropharyngeal areas

(the buccal cavity) or the skin. The relative ease of inactivation of the molecule confers on it the unique safety margin during its use in therapy. The therapeutic regimen of 1,2,4-trioxolane represents a special concept in pharmacodynamics whereby small amounts of the agent is administered to initiate a biological response modification which then develops a cascade of reactions through molecular signaling [2, 3]. The pharmacokinetic behaviour of such an agent would not be expected to provide significant levels of the compound and its metabolites in body fluids for detection and especially due to its rapid rate of metabolism and its non-parenteral mode of administration. This is a novel concept in modern pharmacotherapy.

Storage requirements

The product is stable at ambient temperatures (15°C-30°C). However, the activity progressively reduces as ambient temperatures increase. It is, therefore, recommended to keep the drug in a cool dry place, and it is better if refrigerated. It should not be frozen. Keep free from moisture.

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