

Published Research

Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial

A. Emelyanov*, G. Fedoseev*, O. Krasnoschekova*, A. Abulimity*, T. Trendeleva*, P.J. Barnes#

Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial. A. Emelyanov, G. Fedoseev, O. Krasnoschekova, A. Abulimity, T. Trendeleva, P.J. Barnes. #ERS Journals Ltd 2002. ABSTRACT: Asthma is a chronic inflammatory disease of the airways mediated, at least in part, by leukotrienes and other lipid mediators. Experimental studies have shown that lipid extract of New Zealand green-lipped mussel, *Perna canaliculus*, is effective in inhibiting 5 α -lipoxygenase and cyclo-oxygenase pathways responsible for production of eicosanoids, including leukotrienes and prostaglandins. The aim of this study was to assess its effect on symptoms, peak expiratory flow (PEF) and hydrogen peroxide (H₂O₂) in expired breath condensate as a marker of airway inflammation in patients with steroid-naïve atopic asthma in a double-blind randomised, placebo-controlled clinical trial.

Forty six patients with atopic asthma received two capsules of lipid extract (Lyprinol) or placebo b.i.d. for 8 weeks. Each capsule of lipid extract contained 50 mg ν -3 polyunsaturated fatty acids and 100 mg olive oil, whereas placebo capsules contained only 150 mg olive oil.

There was a significant decrease in daytime wheeze, the concentration of exhaled H₂O₂ and an increase in morning PEF in the lipid extract group compared to the placebo group. There were no significant side-effects.

The authors conclude that lipid extract of New Zealand green-lipped mussel may have some beneficial effect in patients with atopic asthma. *Eur Respir J* 2002; 20: 596–600.

Efficacy and tolerability of mussel-Lyprinol® omega-3-complex on inflammatory rheumatoid disorders

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ABSTRACT

This 12 week drug monitoring study evaluated the effects of Sanhelios mussel-Lyprinol® omega-3-complex, on 50 adult men and women suffering from inflammatory rheumatoid arthritis. 34 of the 50 patients required medicinal treatment before and during the study. Upon completion of the study, for 21 of the 34 subjects (64 %) current drug therapy could be reduced or terminated. 13 of those did not even require further therapy. At the end of the treatment period, 38 % of all subjects were regarded as being free from disorders and the number of subjects suffering from severe pain was significantly decreased from 60% (at baseline) to 25 % (at completion of the trial). A significant positive effect was observed for all investigated parameters. Sanhelios mussel-Lyprinol® omega-3-complex was generally very well tolerated, with only one, non-serious adverse event (mild nausea) observed, which can probably be related to the study medication. Sanhelios mussel- Lyprinol® omega-3-complex , therefore, proved to be an effective and very well tolerated dietary supplement for the treatment of inflammatory rheumatoid arthritis

Clinical Efficacy and Safety of Lyprinol ®, a Patented Extract from New Zealand Green Lipped Mussel (Perna Canaliculus) in Patients with Osteoarthritis of the Hip and Knee : Multicenter Clinical Trial with a 2 Months Treatment Period

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- Abstract- Objective : To validate the clinical efficacy and safety of Lyprinol (a patented extract from

Perna Canaliculus), a **LOX** inhibitor in patients with osteoarthritis.

Methods : In this multicenter trial, 54 patients with symptomatic osteoarthritis of the knee and hip were to receive Lyprinol at a dose of 2 capsules twice a day. After 4 and 8 weeks treatment period, the following parameters were analyzed ; Visual analogue scale, Lequesne index, Global assessment by patients, Global assessment by doctors and Adverse effects.

Results : Lyprinol treatment led to significant improvement in the signs and symptoms of osteoarthritis as determined by all efficacy measures. After 4 and 8 weeks treatment period, 53% and 80% of patients experienced significant pain relief and improvement of joint function. There was no proven adverse effect during this clinical trial.

Conclusion : Lyprinol was very effective and promised anti-inflammatory material to relieve the signs and symptoms of osteoarthritis without adverse effect.

Treatment of knee osteoarthritis with Lyprinol®, lipid extract of the green-lipped mussel – a double-blind placebo-controlled study

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Summary

Treatment of osteoarthritis (OA) includes pain control and improvement of patients' function and quality of life. While conventional treatment such as non-steroidal anti-inflammatory drugs and simple analgesics may achieve these goals, their use is not without side-effects. The use of "natural remedies" and "folklore medicines" is therefore commonly practised by patients with OA. Lyprinol® is a lipid extract of the green-lipped mussel which is rich in omega-3 fatty acids and has previously been shown to have anti-inflammatory effects in both *in vitro* and animal studies. The aim of this study was to compare the effects of Lyprinol® with placebo on the signs and symptoms and patient quality of life in the treatment of knee OA. Eighty patients with knee OA were randomized to receive either Lyprinol® or placebo for six months. All were allowed paracetamol rescue treatment during the study and were reviewed at week 0, 2, 4, 8, 12, 18 and 24 for arthritis assessment and safety evaluation. Assessment of the patients' arthritis included the use of a 100 mm visual analog scale (VAS) for pain, patient's and physician's global assessment of arthritis, a validated Chinese version of the Oxford Knee Score (COKS), a validated Chinese version of the Arthritis Impact Measurement Scale 2-short form (CAIMS2-SF), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Improvement in almost all of the arthritis assessment parameters was observed in both groups of patients studied. However, there was a greater improvement in the perception of pain as measured by the VAS, and patients' global assessment of arthritis in those who took Lyprinol® when compared with controls from week 4 following adjustment for the change in the amount of paracetamol used between study visits. Patients who took Lyprinol® but not placebo also had improved scores in the CAIMS2-SF physical function and psychological status domains from week 4. However, changes in these scores did not differ significantly between the two groups at various study visits. When used over six months, Lyprinol® was safe and well tolerated with no serious side-effects reported. Further, there were no significant differences in the overall incidence of adverse reactions or withdrawal from study as a result of trial drug toxicity between Lyprinol® and placebo treated patients. In conclusion, Lyprinol®, a lipid extract of the green-lipped mussel, may be considered a safe option in the treatment of OA.

The CO₂-SFE crude lipid extract and the free fatty acid extract from *Perna canaliculus* have anti-inflammatory effects on adjuvant-induced arthritis in rats

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The anti-inflammatory (AI) activity of a supercritical fluid extract (CO₂-SFE) of tartaric acid-stabilised *Perna canaliculus* mussel powder, and of the free fatty acid (FFA) class separated from the CO₂-SFE extract by column chromatography, was investigated in the rat adjuvant arthritis model.

Administration of the CO₂-SFE extract (100 mg/kg BW/day s.c.) for 15 days post-adjuvant inoculation significantly reduced rear paw swelling by 34% and the deterioration in total body condition by 52% in arthritic rats, compared to vehicle controls. These observations were accompanied by a decreased serum ceruloplasmin oxidase activity, and reduced inflammatory response of the spleen. The mussel FFA extract given at one third of the dose (30 mg/kg BW/day s.c.) and for a shorter treatment period (5 days during the inflammatory phase) achieved an even greater AI activity, and was equipotent to piroxicam (2 mg/kg BW/day s.c.). Preliminary toxicology assessment using both arthritic and non-arthritic (healthy) rats revealed no significant differences between the mussel treatment groups and respective vehicle controls in either organ weights, tissue histology or selected biochemical parameters. These results indicate the CO₂-SFE crude lipid extract and its FFA components from stabilised *P. canaliculus* mussel powder contain biologically significant AI activity *in vivo*, with no apparent adverse side effects. © 2007 Elsevier Inc. All rights reserved.

Anti-cyclooxygenase effects of lipid extracts from the New Zealand green-lipped mussel, *Perna canaliculus*

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Abstract

Total lipid extracts of *P. canaliculus* (a bivalve marine mollusc native to New Zealand, commonly called the green-lipped mussel) and *Mytilus edulis* (commonly called the common blue mussel) moderately inhibited ovine COX-1 and COX-2 pure enzymes in vitro. The inhibition was increased after the mussel extracts were saponified by KOH hydrolysis. Protease- and protease-lipase-hydrolysed lipid extracts of *P. canaliculus* exhibited similarly strong COX inhibition as the KOH-hydrolysed extract. Lyprinol® (a commercial extract from *P. canaliculus*) also exhibited strong inhibition of both COX isoforms, an effect that was increased 10-fold upon subsequent hydrolysis. In contrast, fish oil was not as anti-COX active as Lyprinol. The Lyprinol free fatty acid fraction, and to a lesser extent the Lyprinol triglyceride fraction, were the only lipid classes of Lyprinol to exhibit strong inhibition of the COX isoforms. The purified PUFA extracts were all bioactive, potently inhibiting COX-1 and COX-2. Incubation of Lyprinol in the absence of exogenous arachidonic acid (AA) showed the appearance of alternate prostaglandin metabolites, confirming Lyprinol PUFA as a competitive substrate inhibitor of AA metabolism. © 2006 Elsevier Inc. All rights reserved.

Novel anti-inflammatory ω -3 PUFAs from the New Zealand green-lipped mussel, *Perna canaliculus*

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The present study has identified in the marine mollusc, *Perna canaliculus*, an homologous series of novel omega 3 polyunsaturated fatty acids (ω -3 PUFA) with significant anti-inflammatory (AI) activity. The free fatty acid (FFA) class was isolated from a supercritical-CO₂ lipid extract of the tartaric acid-stabilised freeze-dried mussel powder by normal phase chromatography, followed by reversed-phase high performance liquid chromatography (RP-HPLC). The RP-HPLC involved separation based on carbon numbers, followed by argentation-HPLC (Ag-HPLC) of the methyl esters based on degree of unsaturation. Identification of the FFA components was performed using gas chromatography (GC) with flame ionisation detection, and individual structures were assigned by GC-mass spectroscopy (GC-MS). Inhibition of leukotriene production by stimulated human neutrophils was used as an in vitro screening method to test the AI activity of the purified PUFAs. A structurally related family of ω -3 PUFAs was identified in the most bioactive fractions, which included C18:4, C19:4, C20:4, and C21:5 PUFA. The C20:4 was the predominant PUFA in the extract, and was a structural isomer of arachidonic acid (AA). The novel compounds may be biologically significant as AI agents, as a result of their in vitro inhibition of lipoxigenase products of the AA pathway. © 2007 Elsevier Inc. All rights reserved.

Lyprinol™: a potential preventive treatment for inflammatory bowel disease (IBD)?

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Background- Fish oil and the stabilised lipid extract of New Zealand Green Lipped Mussel (Lyprinol™; LYP) are considered beneficial in treating arthritis and other inflammatory conditions. Unlike fish oil, it is uncertain whether any benefit seen with LYP is due to its omega-3 (ω 3) fatty acid content. We compared the effect of LYP and fish oil pre-treatments on experimental induction of IBD in mice.

Methods- Male C57BL/6 mice aged 6 weeks were gavaged daily for 13 days with 150 μ l of olive oil (OO, n=7), LYP (5mg in OO; n=8) or fish oil (FO, 55mg EPA/DHA; n=8). Mice consumed 2% dextran sulphate sodium (DSS) for 6 days from day 7 to induce colitis. Body weight and disease activity index (DAI) scores were recorded daily; colonic inflammation was assessed by myeloperoxidase (MPO) activity and histopathologic damage to the ileum and colon.

Results- FO treatment had no significant benefit compared with OO. By day 12 of the trial, OO treated mice had gained 15 \pm 2% body weight, FO treated mice had gained 6 \pm 5% (significantly lower than LYP and OO P<0.05) and LYP treated mice had gained 21 \pm 3% (significantly higher than FO P<0.05): LYP treated mice had a lower DAI score (0 vs. 1 for OO, 4 for FO). Compared with FO, LYP treated mice had smaller crypt area losses (distal colon), lower caecum and colon weights and a trend for lower overall colitis severity in the distal colon. MPO activity was not significantly affected by either LYP or FO vs. OO (see table).

Conclusions- These findings indicate that LYP may be potentially useful in ameliorating symptoms of IBD. The lack of effect of FO indicates that the benefit of LYP is attributable to components of the stabilised lipid extract other than its ω 3 content. A dose-response evaluation of LYP in experimental IBD is warranted.

Lyprinol (stabilised lipid extract of New Zealand green-lipped mussel): a potential preventative treatment modality for inflammatory bowel disease

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Background. Lyprinol (Pharmalink International), the stabilised lipid extract of the New Zealand green-lipped mussel, is currently used to relieve symptoms of arthritis. We investigated the effect of pretreatment with Lyprinol (LYP) on experimentally induced inflammatory bowel disease (IBD) in mice. **Methods.** Male C57BL/6 mice (aged 6 weeks) were gavaged daily for 13 days with (150 μ l) olive oil (OO; n = 7), fish oil (FO; n = 8), or LYP (n = 8). Mice consumed 2% dextran sulfate sodium (DSS) for 6 days, starting on day 7. Body weight and disease activity index (DAI) scores were recorded daily. Colonic damage was determined by histopathology. Colonic inflammation was quantified by myeloperoxidase (MPO) activity. **Results.** LYP treatment significantly (P < 0.05) reduced body weight loss, DAI scores, crypt area losses, and cecum and colon weights, compared with FO treatment. MPO activity was not significantly affected by any treatment. **Conclusions.** These findings provide preliminary evidence that Lyprinol may be potentially useful in ameliorating symptoms of IBD. The benefit, however, is unlikely to be due to the omega-3 fatty acid content. Dose-response evaluation of Lyprinol in experimental IBD is warranted.

Pain Controlling and Cytokine-regulating Effects of Lyprinol, a Lipid Extract of Perna Canaliculus, in a Rat Adjuvant-induced Arthritis Model

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Using an adjuvant-induced arthritis rat model, we investigated the effects of a lipid extract of Perna canaliculus (Lyprinol[®]) on pain. Radiological examinations, as well as levels of pro- and anti-inflammatory (AI) cytokines, were measured aiming to provide independent objective data to the pain controlling investigation. We confirmed the ability of Lyprinol[®] to control pain at the initial phase of its administration; with similar efficacy to that observed with Naproxen. The pain scores slowly increased again in the group of rats treated with Lyprinol[®] after day 9–14. The Naproxen-treated rats remained pain-free while treated. Both Naproxen and Lyprinol[®] decreased the levels of the pro-inflammatory cytokines TNF- α and IFN- γ , and increased that of IL-10. Extra-virgin olive oil was ineffective on cytokine secretion. Rats treated with Lyprinol[®] were apparently cured after 1 year. This study confirms the AI efficacy of this lipid extract of P. canaliculus, its initial analgesic effect, its perfect tolerance and its long-term healing properties.

Gas Chromatography–Chemical Ionization–Mass Spectrometric Fatty Acid Analysis of a Commercial Supercritical Carbon Dioxide Lipid Extract from New Zealand Green-lipped Mussel (*Perna canaliculus*)

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ABSTRACT: Supercritical fluid extracts of New Zealand green-lipped mussels (NZGLM) have been suggested to have therapeutic properties related to their oil components. The large number of minor FA in NZGLM extract was characterized by a GC–CIMS/MS method that excels at identification of double-bond positions in FAME. The extract contained five major lipid classes: sterol esters, TAG, FFA, sterols, and polar lipids. The total FA content of the lipid extract was 0.664 g/mL. Fifty-three unsaturated FA (UFA) were fully identified, of which 26 were PUFA, and a further 21 UFA were detected for which concentrations were too low for assignment of double-bond positions. There were 17 saturated FA, with 14:0, 16:0, and 18:0 present in the greatest concentration. The ten n-3 PUFA detected included 20:5n-3 and 22:6n-3, the two main n-3 FA; n-3 PUFA at low concentration were 18:3, 18:4, 20:3, 20:4, 21:5, 22:5, 24:6, and 28:8. There were 43 UFA from the n-4, n-5, n-6, n-7, n-9, and n-10 families, with 16:2n-4, 16:1n-5, 18:1n-5, 18:2n-6, 20:4n-6, 16:1n-7, 20:1n-7, 16:1n-9, 18:1n-9, and 20:1n-9 being the most abundant. In general, we estimated that FAME concentrations greater than 0.05% (w/w) were sufficient to assign double-bond position. In total, 91 FA were detected in an extract of the NZGLM, whereas previous studies of fresh flesh from the NZGLM had reported identification of 42 FA. These data demonstrate a remarkable diversity of NZGLM FA.

Gastroprotective and anti-inflammatory properties of green lipped mussel (*Perna canaliculus*) preparation.

Rainsford KD, Whitehouse MW.

Freeze-dried powdered preparations of whole (i.e. without shell) green-lipped mussel (*Perna canaliculus*) from New Zealand given orally to rats showed some modest anti-inflammatory activity (carrageenan paw oedema). This material strikingly reduced the gastric ulcerogenicity of several non-steroid anti-inflammatory drugs in rats and pigs. The gastroprotective activity in rats was primarily associated with particular lipid fractions, which exhibited differential protective activity against acetylsalicylic acid on the one hand and indometacin on the other.

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Over the counter (OTC) oral remedies for arthritis and rheumatism: how effective are they?

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Abstract—Background: Increasingly patients resort to alternative remedies for arthritis and rheumatism, perhaps partly impelled by reports of toxicities from prescribed non-steroid anti-inflammatory drugs (NSAID). There is uncertainty about whether the most common alternative treatments provide relief or may cause adverse reactions.

Aim: To ascertain the validity of manufacturers' claims permitted by the Therapeutic Goods Administration (TGA) in Australia for a range of self-medication products to treat the pain and inflammation of arthritis, available in local pharmacies, supermarkets or by mail order and in other countries.

Methods: OTC products were administered orally to rats in standard assays for suppressing experimental arthritis and fever and for determining potential gastrotoxicity.

Results: The three NSAIDs available OTC were efficacious but gastrotoxic. Of the 37 herbal formulations examined, seven were as effective as ibuprofen in the anti-arthritic assay without causing gastric bleeding. Five of the 10 animal-sourced products tested were also effective without evident toxicity. Within a certain class of product, e.g. celery seed extracts or dried mussel preparations, efficacies ranged from almost zero to highly effective.

Conclusions: Consumers currently have no guide to the likely efficacy of TGA-approved remedies. Quality control is urgently needed to justify the veracity of TGA-permitted and other claims on product labels.

LYPRINOL INHIBITS LTB₄ PRODUCTION BY HUMAN MONOCYTES.

B. Dugas(1)

Summary

The effect of Lyprinol was evaluated on LTB₄-induced human monocytes (normal and allergic donors) Activation. Peripheral blood normal monocyte-derived monocytes when stimulated by Interleukin- 4 (IL-4) produced high amounts of leukotriene B₄ (LTB₄) through the activation of the 5-Lipoxygenase Pathway. Maximal effect was observed in the presence of 10ng/ml IL-4, and maximal LTB₄ production was reached 40 min after the onset of stimulation. When stimulated for 48 h with IL-4, Resting human monocytes expressed and released the low affinity receptor for LgE (CD23), and were inhibited in the presence of Lyprinol, or of the non redox 5-lipoxygenase inhibitor (BWB70C), Suggesting that the production of LTB₄ partially contributed to the IL-4-induced CD23 Expression and release. In addition to these Phenotypical changes, IL-4 Primed the phorbo-12-myristate-13 acetate (PMA)-indu-ced luminol- dependent chemiluminescence response (LDCL) by normal human monocytes; this priming effect was abrogated in the presence of Lyprinol, or BW B70C. Monocyte-derived monocytes from allergic patients spontaneously produced high amounts of LTB₄, exoressed CD23 expression, and had an increased oxidative metalbolism. In the presence of Lyprinol, or of a BW B70C, the hyper- activation of monocytes from allergic patients was significantly suppressed. Taken together, these data indicated that the pharmacological control of the 5-lipoxygenase pathway in human monocytes can be achieved with regulate the expression and release of CD23 and the respiratory burst of human monocytes.

The treatment of arthritis with a lipid extract of perna canaliculus: a randomized trail

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SUMMARY. Objective: to assess the efficacy of lipid extract of the New Zealand green-lipped mussel, *Perna canaliculus* in rheumatoid and osteoarthritis ad compare it with green-lipped muscle powder. Design: A double – blind 3 month parallel comparison of the two preparations and a further 3- month period on lipid extract for all patients Setting: The out-patient department of the Glasgow Homoeopathic Hospital. Interventions; Stabilized green-lipped mussel powder , 1150 mg/day and the derived lipid extract, 210 mg/day. Main Outcome Measures: Articular index of joint tenderness (AI), morning stiffness (limbering-up-time, LUT), grip strength in each hand, visual analogue scale of pain (VA) and functional index (FI). Results: Seventy six percent of rheumatoid and 70% of osteoarthritis patients benefitted. AI, LUT and FI improved significantly by 3 months. The two preparations appeared equally efficacious. One patient experienced fluid retention and one developed nausea. There were no other adverse reactions. Conclusion: Both the stabilized freeze-dried mussel powder and its derived lipid extract are effective in the reducing pain, swelling and stiffness and in improving functional index in rheumatoid arthritis and osteoarthritis.

The Effect of a Lipid Extract of the New Zealand Green-Lipped Mussel in Three Cases of Arthritis

SHEILA L.M. GIBSON., M.D., B.Sc., M.F.HOM.

INTRODUCTION

Extracts of the New Zealand green-lipped mussel, *perna canaliculus*, have been used since the 1980s for the treatment of both rheumatoid arthritis and osteoarthritis. A pilot study suggested that this could be helpful and a double-blind placebo-controlled trial found the mollusk to be effective for 68% of patients with rheumatoid arthritis and for 40% of those with osteoarthritis (Gibson, et al., 1980). Not all researchers, however, obtained similar beneficial results (Caughey, et al., 1983; Huskisson, et al., 1981; Larkin, et al., 1980s that stabilization of the product was necessary to maintain biologic activity. More recently, a lipid extract has been developed and has proved to be indistinguishable in its activity of thus preparation is in the lipid fraction rather than in the protein moiety (Gibson and Gibson, 1998). In the comparative study of the lipid fraction versus the stabilized mussel powder (Gibson and Gibson, 1998), statistically significant improvements were observed in patients with both rheumatoid arthritis and osteoarthritis within 1 month of starting treatment, but there was no obvious overall difference between the two preparations. Seventy-five percent of patients with both rheumatoid arthritis and osteoarthritis benefitted. Although the number of patients in the trial was small (60 in total; 30 with rheumatoid arthritis and 30 with osteoarthritis), some patients, particularly those with rheumatoid arthritis, seemed to respond remarkably quickly to the lipid extract as illustrated by the following three case studies. These three patients was monitored by means of the articular index (AI) of joint pain (Ritchie, et al., 1968), Limbering up time (LUT) or morning stiffness, grip strength in each hand (Lee, et al., 1973), which are the answers to a series of 22 questions that assess each patient's ability to turn the head, bend, use the hands and arms, and walk and climb up and down stairs. The values obtained for these parameters for the three patients at the first consultation and after 2 months, are given in Table 1.

The Anti-inflammatory Effects of omega Tetraenoic Fatty Acids Isolated from a Lipid Extract from the mussel, *Perna canaliculus*

T.A Macrides, *Perna canaliculus*. T.A. Macrides(1), A.P. Treschow(1), N. Kalafati(1), P.F.A. Wright(1), P.M. Wynne(2)

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The lipid extracts of *Perna canaliculus*, the green lipped mussel of New Zealand, have been shown to have anti-inflammatory activity (AI) and the greatest activity is shown by the polyunsaturated free fatty acid (PUFA) class(1). We have identified a homologous series of novel omega PUFA's with significant AI activity in an in vitro leukotriene inhibition assay. The proportion of novel PUFAs in the crude lipid extract was 0.12% w/w and in the total mussel material, 0.005% w/w. The total free FAs were isolated from a supercritical-CO₂ lipid extract of the freeze-dried mussel powder by flash chromatography, followed by reversed-phase HPLC. This technique involved a separation based on carbon number, followed by Ag-HPLC of the methyl esters based on the degree of unsaturation. Identification of FA components was performed using GLC with flame ionisation detection and individual structure were assigned from the pyrrolidide derivatives by GC-MS. Inhibition of leukotriene B₄ (LTB₄) produced from stimulated human neutrophils was used as an in vitro screening method to test the efficacy of purified PUFAs for AI activity. The two most active fractions obtained from the separations inhibited LTB₄ formation by 64% and 47% respectively (at 1:100 dilution). A structurally related family of Omega free fatty acids was identified in the most bioactive fraction which included C18:4, C19:4 and C20:4 PUFAs. Two C20:0Omega3 structural analogue of arachidonic acid have been synthesised for testing in an in vivo adjuvant-induced polyarthritis model 1. T.A. Macrides and N. Kalafatis (1995) Int. Patent PCT/AU 95/004/85.

Lipid, FA, and Sterol Composition of New Zealand Green Lipped Mussel (*Perna canaliculus*) and Tasmanian Blue Mussel (*Mytilus edulis*)

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Abstract: The lipid FA, and sterol composition of the New Zealand green lipped mussel (NZGLM), *Perna canaliculus*) and of the Tasmanian blue mussel (TBM, *Mytilus edulis*) were com-pared using TLC-FID and GC-MS. The respective mussel species were obtained from three different sites in both New Zealand (NZ) and Tasmania, Lipid class distribution of both mussel species was characterized by a high proportion of phospholipid (PL, 57-79%) and TG (10-25%), FFA (7-12%) and sterols (ST, 12-18%). The NZGLM had higher Proportions of TG, FFA and ST ($P < 0.01$), where as the TBM had a higher proportion of PL ($P < 0.01$). There were higher proportions of total PUFA, saturated FA, n-3 FA, in the NZGLM were 16:0 (15-17%) 20:5n-3 (14-20%), and 22:6n-3 (11-17%). The same FA dominated Lipids in the TBM, although there were significantly higher proportions of 16:0 ($P = 0.000$) and 22:6 n-3 ($P = 0.003$) and lower proportions of 20:5n-3 ($P = 0.0072$) in the TBM. A novel PUFA, 28:8n-3, was detected in both mussels with higher amounts in the TBM, which probably reflects a greater dietary contribution if dinoflagellates for this species. Cholesterol was the dominant sterol in both mussels. Other major sterols included brassicasterol, 22 methycholesterol, trans-22-dehydrocholesterol, and desmosterol. There were significant differences ($P < 0.05$) between the NZGLM and TBM for the 12 of 20 sterols measured. Six sterols showed significant site clifferrnces for the NAGLM, and 10 for the TBM. The difference in the FA and sterol composition between the two species may be due to the diet of the NZGLM being more diatom-derived and the diet of the TBM having a greater dinoflagellate compent. Paper no.L8863 in *Lipids* 37, 587-595 (June 2002).

Anti-inflammatory effects of stabilized lipid extract of *Perna canaliculus* (Lyprinol®)

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Conclusion

Lyprinol is an extraordinary, and promising natural product. It is totally safe, easy to take, and does not impair the normal function of our organism. It demonstrates a gastro protective effect. Whereas most NSAIDs are gastro erosive. Contrary to fish oils, Lyprinol does not affect the thrombosis-associated system in normal individuals, i.e, does not promote bleeding.

It is remarkably active on the diverse components of inflammation, with soecific cellular, mucosal, or joint targets. Much remains to be discovered and we are planning many studies addressing the too many ailments that make humans, even more the aging ones, suffer and complain.” It is the medicine of the future “(Micheal W. Whitehouse).

LYPRINOL : ANTI-INFLAMMAORY AND UThERINE-RELAXANT ACTIVITES IN RATS, WITH SPECIAL REFERENCE TO A MODEL FOR DYSMENORRHOEA

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Summary

Lyprinol exhibits anti-inflammatory activity distinct from that of most NSAIDs, controlling chronice but not acute inflammation. Unlike Cox-1 inhibitors (aspirin, meclofenamic acid) it is not gastro-toxic.

Predosing rats with Lyprinol can modify both (i) the spontaneous and (ii) the ocytocin-induced contractions of the uterus. In humans there is anecdotal evidence that Lyprinol can relieve dysmenorrhea.

This report explores the concept that the uterotrophic actions of Lyprinol are conditioned by:
. the intrinsic profile of estrogenic hormones an progestagens and,
. certain extrinsic stimuli

Evidence from in vitro studies indicates that Lyprinol is not a smooth muscle relaxant and that its uterotrophic mechanism is not that of cyclo-oxygenase inhibitor, but may mimic that of a leukotriene receptor antagonist

ANTI-INFLAMMATORY ACTIVITY OF LIPID FRACTION (LYPRINOL) FROM THE NZ GREEN-LIPPED MUSSEL

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ABSTRACT

Whitehouse MW, Macrides TA, Kalafatis N, Betts WH, Haynes DR Broadbent J. Anti-inflammatory activity of a lipid fraction (Lyprinol) from the NZ green-lipped mussel. *Inflammopharmacology*. 1997;5:237-246.

A lipid-rich extract, prepared by supercritical fluid extraction of fresh stabilized mussel powder (Lyprinol), showed significant anti-inflammatory (AI) activity given therapeutically and prophylactically to Wistar and Dark Agouti rats developing either (a) adjuvant-induced polyarthritis or (b) collagen(II)-induced autoallergic arthritis, with ED₅₀ < 15 mg/kg; c.f. naproxen > 25 mg/kg or various therapeutic oils (flaxseed, evening primrose, fish) > 1800 mg/kg given orally. Lyprinol showed little or no activity in acute irritation assays (carrageenan, kaolin, histamine) indicating it is not mimicking rapid-acting NSAIDs. Incorporating Lyprinol into arthritogenic adjuvants composed of heat-killed Mycobacterium tuberculosis suspended in olive oil or squalane, effectively prevented arthritis development at a dose of 5 mg/rat. By contrast, 'dummy adjuvants' prepared with Mycobacterium tuberculosis and flaxseed, evening primrose or fish oils were still arthritogenic in Dark Agouti rats (doses of oil = 90 mg/rat). Lyprinol subfractions inhibited leukotriene-B₄ biosynthesis by stimulated human polymorphonuclear leukocytes in vitro, and prostaglandin-E₂ production by activated human macrophages in vitro. Much of this AI activity was associated with polyunsaturated fatty acids and natural antioxidants (carotenoids, etc.). In contrast to NSAIDs Lyprinol is non-gastrotoxic in disease-stressed rats at 300 mg/kg po and does not seem to affect platelet aggregation (human, rat). These data show Lyprinol to be a reproducible, relatively stable, source of bioactive lipids with much greater potency than plant/marine oils currently used as nutritional supplements to ameliorate signs of inflammation.

Keywords: NZ green-lipped mussel, Lyprinol, lipid fraction, inflammation, NSAIDs

STEROID-SPARING ACTION OF LYPRINOL, A LIPID FRACTION FROM THE NZ GREEN-LIPPED MUSSEL.

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Corticosteroids (CS) are powerful anti-inflammatory (AI) agents but their adverse effects on lymphoid and connective tissues largely restrict their clinical use. Lyprinol is a salt-free, non-allergenic AI nutraceutical that inhibits lipoxygenases and is not lympho-toxic (Whitehouse et al 1997). In one case of asthma, Lyprinol ingestion reduced prednisone requirement by 80% (Harbison and Whitehouse 2000), suggesting a synergistic effect of CS with Lyprinol. This possible synergy was examined using rat models of chronic inflammation initiated by three clinically relevant pathogenic irritants namely yeast cell wall (zymosan 0.5 mg), silica (4 mg), and calcium pyrophosphate (causing pseudo gout, 7 mg), each injected in 0.2 ml saline/rat paw. After three hours animals were dosed orally and daily thereafter for four days. Drug efficacy/toxicity was assessed by a) changes in paw thickness and body weight measured daily and b) size of spleen and thymus (day 5) and susceptibility to gastric bleeding from NSAIDs (60 mg/kg ibuprofen po or 5 mg/kg piroxicam ip) given on day 5 after fasting overnight. Prednisone (P) and dexamethasone (D) used singly gave ED50s for reducing paw swelling (after 48 hours) of approximately 6 mg/kg and 0.5 mg/kg, respectively, with massive thymus involution (>90%) and considerable NSAID-gastropathy, compared to non-CS-treated inflamed rats. By contrast, repeated dosing with aspirin (300 mg/kg), ibuprofen (60 mg/kg), naproxen, Celebrex and Vioxx (all at 25 mg/kg) had almost no effect on persisting paw swelling. Minimally effective (<ED20) CS doses of 3 mg/kg P or 0.1 mg/kg D given alone now became >ED70 doses when co-administered with Lyprinol 13 mg/kg, zafirlukast 20 mg/kg) were not able to replace Lyprinol in these rat studies. Commercial extracts of whole green mussel (*Perna canaliculus* from MacLab Australia) 260 mg/kg also acted synergistically with P and D but similar extracts from NZ-sourced blue mussel (*Mytilus edulis*) did not. These data confirm that the lipoxygenase-inhibiting and other AI properties of Lyprinol allow considerable reduction of CS dosage when used concurrently to treat chronic/fibrogenic inflammation.

Harbison SJ and Whitehouse MW (2000) *Med J Aust*, 173,560 Whitehouse MW et al (1997) *Inflammopharmacol*, 5,237-246

Poster-2-16

THE MARINE OIL, LYPRINOL®, IS A SUBSTRATE FOR THE 5-LIPOXYGENASE ENZYME IN PORCINE NEUTROPHILS.

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Lyprinol® is the supercritical fluid oil extract of the New Zealand green-lipped mussel, *Perna canaliculus*. Lyprinol inhibits the production by human neutrophils of leukotriene B(4) (LTB(4)), a significant metabolite of the 5-lipoxygenase (5-LO) enzyme pathway, and has also exhibited anti-inflammatory effects in animal models of arthritis (Whitehouse et al 1997). This study in Porcine neutrophils investigates the inhibition of LTB(4), its isomers (6-trans LTB(4) and 6-trans-12-epi LTB(4)) and 5-hydroxyeicosatetraenoic acid (5-HETE) as well as following the production of Lyprinol-derived 5-LO metabolites. Porcine neutrophils (2.6×10^6 cell/ml) were isolated on a percoll gradient and preincubated (37°C) with various concentrations of Lyprinol (10, 3.3, 1.0, 0.33, 0.1 µg/ml) before addition of arachidonic acid (AA) substrate (2.5µM). The 5-LO pathway was initiated by the addition of calcium ionophore A23187 (2.5µM) and incubated for 5 min. The 5-LO metabolites were identified by comparison of retention times with known standards and quantified against internal standards (prostaglandin B(2) and 15-HETE) by HPLC with photodiode array detection at 270nm (LT's) and 235nm (HETE's). Lyprinol (10µg/ml) inhibited the production of LTB(4) by 72.2₋+1.9%, 6-trans LTB(4) by 47.3₋+2.2%, 6-trans-12epi LTB(4) by 39.0₋+2.1% and 5-HETE by 19.6₋+ 8.7% (n=4), when compared to the vehicle control. Lyprinol itself contains eicosapentaenoic acid (EPA) which the neutrophils converted to similar amounts of LTB(5) and 5-hydroxyeicosapentaenoic acid (5-HEPE) both in the presence or absence of exogenous AA. Other 'leukotriene-like' Peaks exhibiting the characteristic triene chromophore were also produced, but remain unidentified. Therefore in addition to Lyprinol's inhibition of the production of proinflammatory LTB(4), this study has shown that omega-3 polyunsaturated fatty acids present in Lyprinol are substrates for 5-LO. This may contribute to Lyprinol's beneficial effects, as the metabolites are expected to be less proinflammatory than AA metabolites.

Whitehouse MW et al (1997) *Inflammopharmacology*, 5,237-246

Treating inflammation: some (needless) difficulties for gaining acceptance of effective natural products and traditional medicines

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Abstract- The quality of so-called 'natural medicines' is extraordinarily variable. Lack of resolute pharmacological assays contributes to this hiatus. More stringent evaluation of anti-inflammatory and anti-pyretic activities in rats can help resolve some of the uncertainties surrounding (a) preparations of some herbal products including so-called nature's aspirin (e.g. willowbark, ginger), cat's claw, celery seed etc, and (b) some animal lipids (e.g. Lyprinol® (NZ mussel), emu and fish oils). These animal products can be a remarkable resource for supplementing conventional/allopathic therapy for inflammatory disease, e.g. providing lipoxygenase inhibitors. Beyond the verifiable science, the healing professions and the general public still need to examine more carefully criteria for QUALITY(S) in any alternative medicine-to ensure the good (=both reputations and products) are not destroyed by the bad-in essence counteracting Gresham's Law which states: the bad tends to displace the good.