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

Georges M. Halpern, M.D., Ph.D., D.Sc.
Professor Emeritus of Medicine, University of California, Davis
Visiting Professor, The University of Hong Kong

Introduction

The Maoris who live in New Zealand have claimed for centuries that consuming local green-lipped mussels helps them maintain good health. Recent statistics show that the reported incidence of arthritis is extremely low in the coastal-dwelling Maoris, who consume large amounts of green-lipped mussels, whereas Maoris who reside in the interior have the same incidence of arthritis as New Zealanders of European origin. To investigate its reported anti-inflammatory activity, researchers in the United Kingdom, Australia and Japan have studied the effects of various oral preparations of the New Zealand green lipped mussel, *Perna canaliculus*.

Clinical studies since 1980 have shown that a stabilized lipid extract of *Perna canaliculus* is effective in relieving symptoms of rheumatoid arthritis and osteoarthritis. This extract exhibits significant anti-inflammatory effects and has been shown to down-regulate the lipoxygenase pathways responsible for production of pro-inflammatory leukotrienes and other eicosanoids. This article reviews recent research on green lipped mussel extracts as well as technical issues surrounding the development of stable mussel preparations.

Early Clinical Trials

A clinical trial was conducted at Glasgow's Homeopathic Hospital in Scotland by Drs. Robin and Sheila Gibson using capsules of powdered *Perna canaliculus* in patients with arthritis according to ARA criteria. Their study found that:

- 34.8% of the patients experienced considerable improvement,
- 32.6% were helped, but to a lesser extent,
- 32.6% did not improve.

The extract was found to be safe and well tolerated. Many patients reported that it took 3-4 weeks or more to notice a positive effect. Drs. Gibson concluded from this open study that the "extract from the New Zealand green-lipped mussel, *Perna canaliculus*, was a safe and effective alternative nutritional supplement approach for rheumatoid arthritis patients, and for some people plagued with osteoarthritis".

In 1980, groups from the Homeopathic Hospital and the Department of Surgery, Victoria Infirmary, Glasgow, Scotland, reported the results of a double-blind study involving 66 outpatients, 28 with rheumatoid arthritis and 38 with osteoarthritis. All had been

scheduled for surgery to improve their joint conditions; all had failed to respond to conventional treatments.

Patients were randomized with group 1 receiving the mussel extract and group 2 a placebo (dried fish meal powder). Final evaluation was done at day 90. Then all patients were given the mussel extract for 3 months. Regular checks were done on joint stiffness, limbering up time, grip strength, articular index of joint tenderness, pain, functional efficiency, and time to walk 15 meters. Results showed that 68% of RA and 39% of OA patients experienced improvement. Ten percent of patients given the mussel extract experienced a transient aggravation of their symptoms. There were no side effects. The authors concluded: "The extract of New Zealand green-lipped mussel, *Perna canaliculus*, is an effective supplement or possible alternative to other therapies in the treatment of both rheumatoid arthritis and osteoarthritis. It reduces the amount of pain and stiffness, improves the patient's ability to cope with life, and apparently enhances general health. Added to these benefits is the low incidence of side effects. It would therefore seem that the green-lipped mussel extract could be of considerable value to patients suffering from these two chronic and disabling conditions."

Stability and Anti-inflammatory Activity

In 1982, researchers studying the effects of the mussel extract experienced considerable variations from batch-to-batch in the level of anti-inflammatory activity due to poor stability. In 1983, one of these researchers, Professor Takuo Kosuge, of Shizuoka University College of Pharmacy, Japan, developed a process using tartaric acid that helped stabilize the freeze-dried extract. This stabilized freeze-dried extract exhibited little or no variation in anti-inflammatory activity. To further investigate the relationship between stability and anti-inflammatory activity, Michael W. Whitehouse, PhD, conducted *in vivo* studies in rats using an experimental polyarthritis model. At the Princess Alexandra Hospital, Brisbane, Australia, he fed rats for 4 days either with the stabilized, or not stabilized extract. The results (Table 1.) showed that stabilized green lipped mussel extract was dramatically more effective than non-stabilized extract in inhibiting the experimentally-induced inflammatory swelling.

-TABLE 1-

ANTI-INFLAMMATORY ACTIVITY OF NEW ZEALAND GREEN-LIPPED MUSSEL EXTRACTS IN EXPERIMENTAL POLYARTHRITIS MODEL

Dosage given orally for 4 Days (days 10 – 13)

TREATMENT	% INHIBITION OF SWELLING	CLINICAL EFFECTIVENESS	
		TRIAL 1	TRIAL 2
MUSSEL EXTRACT *(STABILIZED)*	100%	71%	83%
MUSSEL EXTRACT *(NOT STABILIZED)*	14%	0%	7%

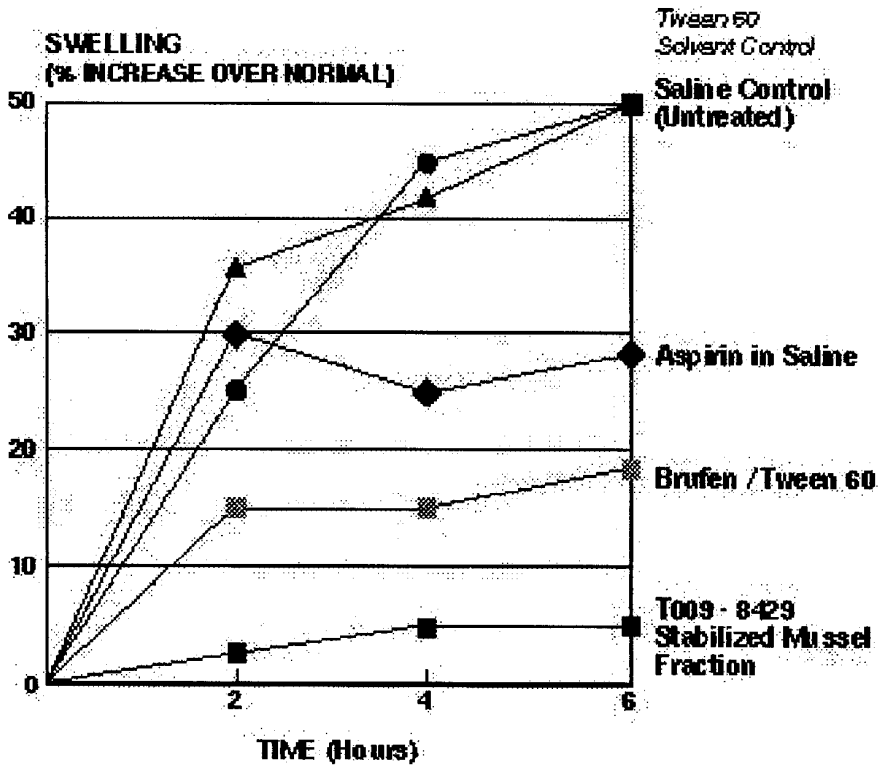
Table 1. Effects of stabilized and non-stabilized *Perna canaliculus* extract given orally for 4 days (days 10 – 13) following experimentally induced inflammation.

In 1983, Theo Macrides, PhD, joined the group of Robert Borland, Ph.D., at Royal Melbourne Institute of Technology in Australia. He separated and characterized the active anti-inflammatory fractions from the stabilized freeze-dried extract of *Perna canaliculus*. Using state-of-the-art technology, including high performance liquid chromatography, nuclear magnetic resonance spectroscopy, and UV-Vis spectroscopy, Dr. Macrides identified a particular lipid fraction that exhibited marked anti-inflammatory activity.

Using a modification (Myers 1984) of the biological test method described by Miller and Ormrod (1981), Macrides compared the effects of aspirin, ibuprofen and stabilized freeze-dried mussel extract in carageenan-induced inflammation in rodents. Results showed that aspirin reduced inflammatory swelling by 40%, ibuprofen by 60% and stabilized mussel extract by 90% (Figure 1).

-FIGURE 1-

COMPARISON OF ANTI-INFLAMMATORY SUBSTANCES



(Figure 1.) Vertical axis represents the extent of the swelling that occurred; the horizontal axis represents the time measurements of swelling taken. Each point

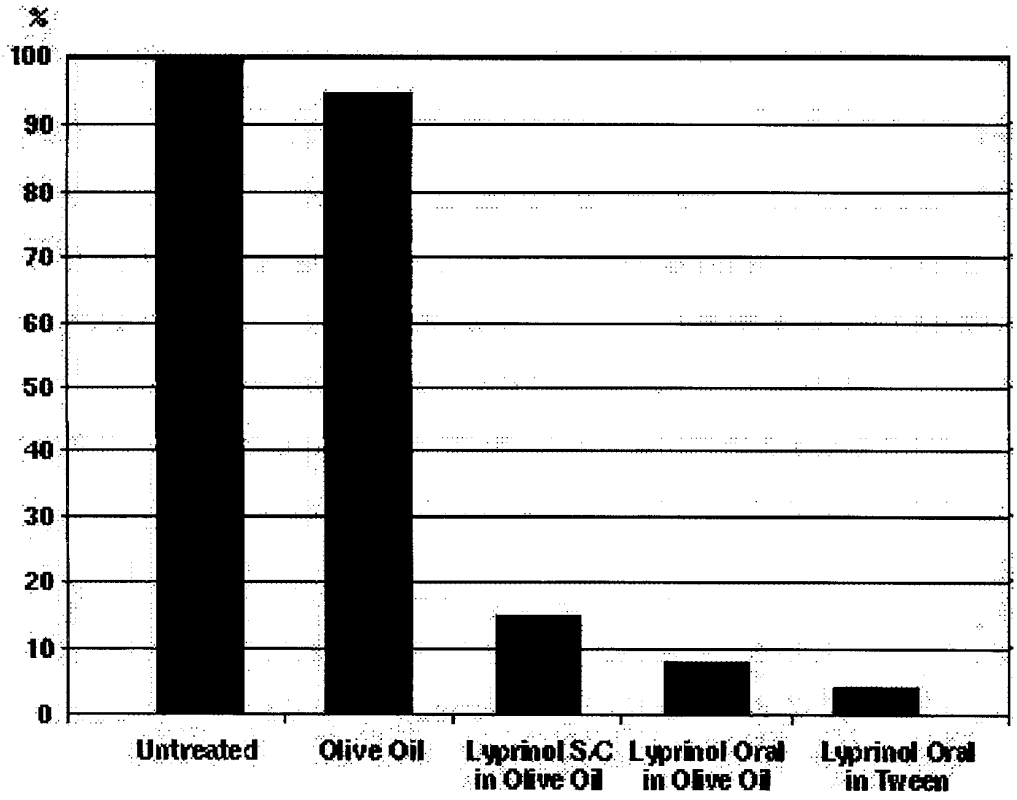
represents the mean of six replicate samples. Fraction T009-8429 chemical nature is lipid and non-steroidal. The effects shown on Fig. 1 were dose-dependent, and increased in potency with each refinement test.

In 1993, a unique method was developed for obtaining a lipid-rich extract of *Perna canaliculus*. It was a dark yellow oil with strong ultra-violet absorbing characteristics, which exhibited substantial anti-inflammatory activity. The chemical nature and structure of this major anti-inflammatory constituent was finally characterized using both gas chromatography and mass spectrometry. This lipid fraction, identified as Lyprinol, was been found to contain a unique combination of triglycerides, sterol esters, free fatty acids, polar lipids, and carotenoids, but no solvent residues. It is obtained by supercritical fluid extraction (SFE) of the stabilized freeze-dried mussel powder using liquefied carbon dioxide. Representing about 4-5% original weight of the freeze-dried mussel powder, it contains no solvent residue as a result of the solvent-free SFE process.

Dr. Whitehouse compared the effectiveness of Lyprinol with other anti-inflammatory and anti-arthritic compounds, using a well-documented model of adjuvant (*Mycobacterium tuberculosis*)-induced polyarthritis . In the first series of experiments, he showed that Lyprinol was effective both orally and by subcutaneous injection (Figure 2). Lyprinol reduced swelling by 88% when administered subcutaneously, and by 93-97% when delivered orally. Other studies confirmed a remarkable topical activity.

-FIGURE 2-

ANTI-INFLAMMATORY EFFECTS OF LYPRINOL GIVEN BY DIFFERENT ROUTES



(Figure 2.) Relative effectiveness of different oils at reducing inflammation as measured by percent reduction in swelling.

The next series compared the effects of a number of commercially available dietary oils with Lyprinol in controlling adjuvant-induced polyarthritis in the model. Lyprinol was given at 1/100th of the dose of the other products. The results demonstrated that Lyprinol is over 200 to 350 times more potent than other oil products tested in preventing the swelling associated with adjuvant-induced polyarthritis.

-TABLE 2-

THE EFFECT OF OILS GIVEN PROPHYLACTICALLY TO PREVENT SWELLING ASSOCIATED WITH ADJUVANT-INDUCED POLY-ARTHRITIS

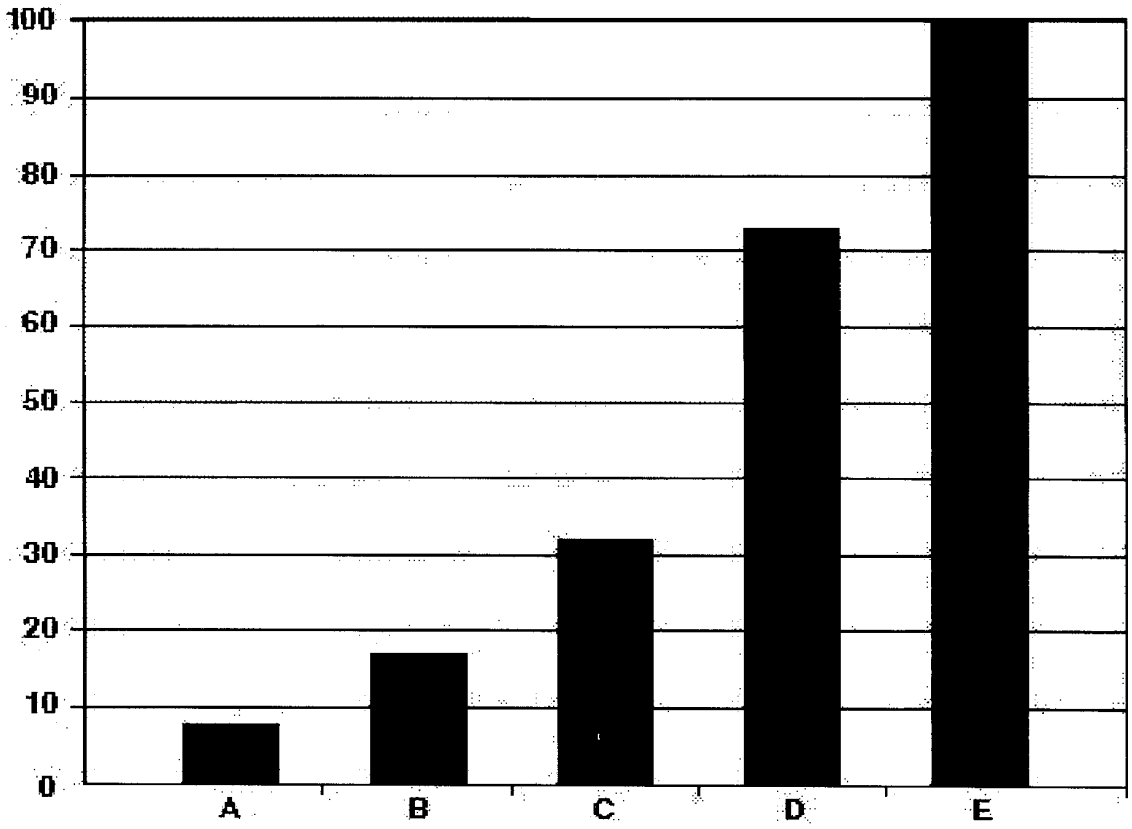
TYPE OF OIL		DOSE (MG/KG)	PERCENT EFFECTIVENESS
FLAX OIL		2000	2%
EVENING PRIMROSE OIL		2000	25%
NORWEGIAN SALMON OIL		2000	32%
EPA FISH OIL		2000	50%
LYPRINOL		20	79%

In the third series, Lyprinol or indomethacin were administered orally for 4 days, and measurement of inflammatory swelling was taken on day 14, 10 days after the initiation of adjuvant-induced polyarthritis. The results are shown on Table 3, and illustrated on Figure 3.

-TABLE 3-
ARTHRITIC INFLAMMATION IN RATS
(Measured as increased rear paw thickness)

Rats were seen with first signs of arthritis 10 days after inoculating rear paw with mycobacterial arthritogen. Dosage was administered orally for 4 days only, then signs of Arthritis were measured on day 14.

TREATMENT	DOSAGE (MG/KG)	% REDUCTION IN INFLAMMATION
A. DIETARY MARINE LIPIDS (LYPRINOL)	5	91%
B. INDOMETHACIN**	5	83%
C. INDOMETHACIN	3	68%
D. INDOMETHACIN	1	26%
E. NO TREATMENT	0	0%



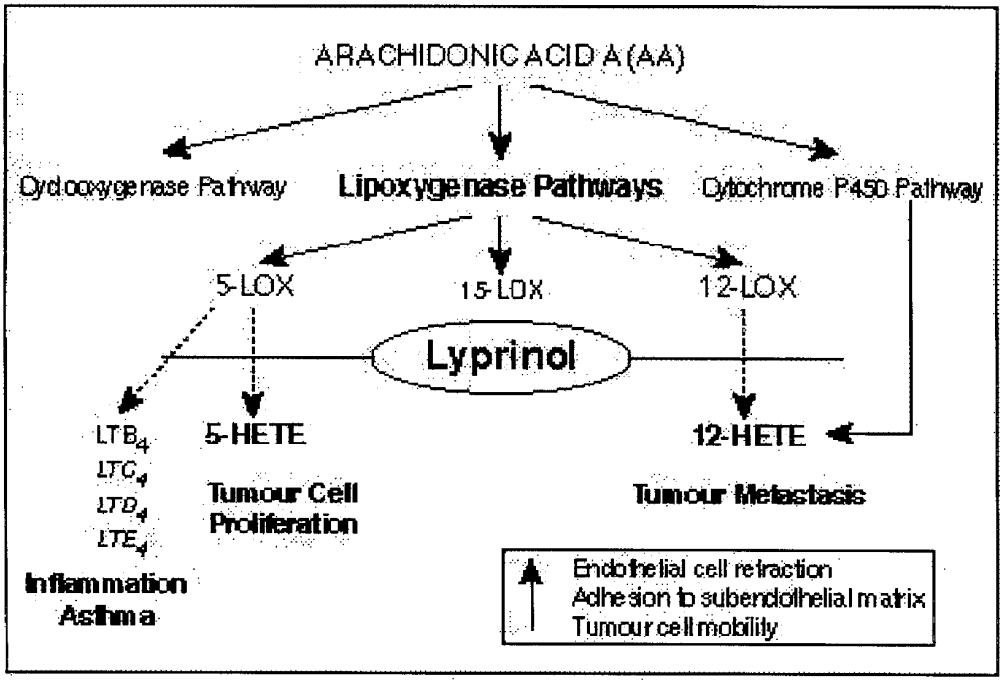
Conclusion:

1. **DML (Lyprinol) is more effective than Indomethacin in this model.**
2. **Stabilized mussel extract is vastly superior to unstabilized mussel extract.**

Mechanism of Action

The metabolism of arachidonic acid via the 5- lipoxygenase pathway of leukocytes leads to the formation of leukotriene B_4 (LTB_4) and the leukotrienes C_4, D_4 and E_4 (LTC_4, LTD_4 and LTE_4) as shown in Figure 4. LTB_4 is a potent chemotactic agent and is responsible for the increased number of leukocytes at sites of inflammation. LTC_4, LTD_4 and LTC_4 are very potent broncho-constricting agents produced by eosinophils in the lung, and whose production is increased in asthma.

-FIGURE 4-
Inhibition of the Lipoxygenase Pathways by Lyprinol



Another major metabolite of the 5-lipoxygenase pathway is 5-hydoxyeicosatetraenoic acid (5-HETE), but until recently there were no known major physiological roles for 5-HETE. However there are now a considerable number of studies which persuasively demonstrate a role for not only 5-HETE but also other HETES, in particular 12-HETE in tumor proliferation and metastases, as shown in Figure 4. In general these studies have demonstrated (i) increased production of 5-HETE and 12-HETE (and to a lesser extent 15-HETE) in tumor cells compared to normal tissues, (ii) that 5-HETE stimulates tumor cell proliferation, and (iii) 12-HETE promotes tumor metastasis.

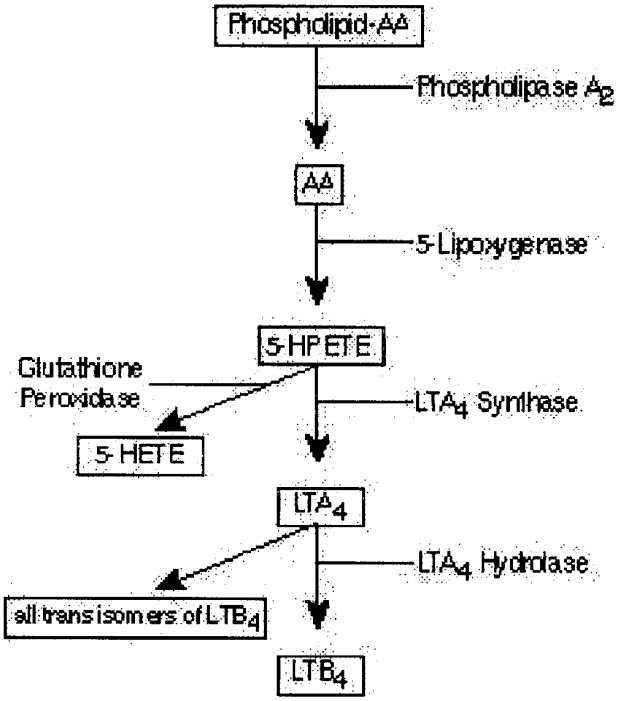
The evidence that Lyprinol inhibits the 5- and 12- lipoxygenase pathways are presented below.

Measuring the activity of the 5- lipoxygenase pathway

The Rheumatology Unit at The Queen Elizabeth Hospital has considerable expertise in the biochemistry and pharmacology on the 5-lipoxygenase pathway, and in particular the production of leukotrienes by human polymorphonuclear leukocytes (PMN or white blood cells), as these are the cells that are initially recruited to an inflammatory site. The principal steps of the 5-lipoxygenase pathway of these cells is shown in Figure 5. In this pathway, arachidonic acid (AA), from membrane phospholipids, is released via the action

of phospholipase A_2(PLA_2). This AA is then substrate for the first enzyme in the pathway - 5 lipoxygenase, which converts it to 5-hydroperoxyeicosatetraenoic acid (5-HPETE). 5-HPETE is then converted enzymatically to either 5-hydroxyeicosa-tetraenoic acid (5-HETE) by glutathione peroxidase, or to leukotriene A_4 (LTA_4) by LTA_4 synthase. LTA_4 is then converted either non-enzymatically to the all trans isomers of LTB4, or hydrolyzed by LTA_4 hydrolase to leukotriene B_4 (LTB_4). Human PMN do not significantly metabolize LTB_4 any further, although other cells, such as eosinophils convert it to the potent vasoconstrictor peptido-leukotrienes, SRS-A.

-FIGURE 5-
The 5-Lipoxygenase Pathway of Human Neutrophils



The lipoxygenase pathways (Leukotrienes AND HETE) Assay

The HPLC assay readily quantifies 5-HETE, 12-HETE, LTB_4, and the two all trans-isomers of LTB_4, and thus gives quantitative data on the relative activities of the enzymes in the 5-lipoxygenase of neutrophils, as well as data on the 12-lipoxygenase pathway of platelets. Thus it is an ideal system to test potential inhibitors of these pathways.

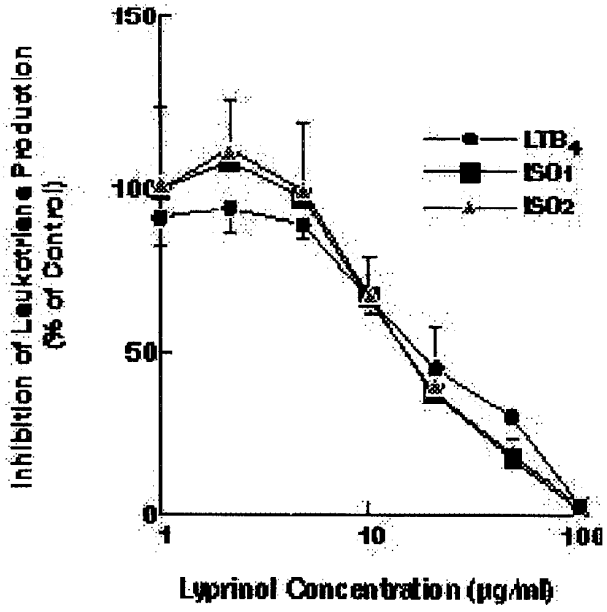
The effects of inhibitory compounds may be tested on isolated human PMN and platelets, in which the pathways are activated by treating the PMN, or platelets, with arachidonic acid, and the calcium ionophore A23187. The addition of arachidonic acid eliminates the PLA_2 step, and provides high levels of substrate for the pathway. Furthermore, such activation is known to maximally drive the pathway to produce the greatest synthesis of

all the metabolites, and thus is also the least sensitive to inhibition. Hence, compounds that do inhibit the pathway activated in this fashion are potentially potent inhibitors.

Inhibition of neutrophil leukotriene and 5-HETE production by Lyprinol

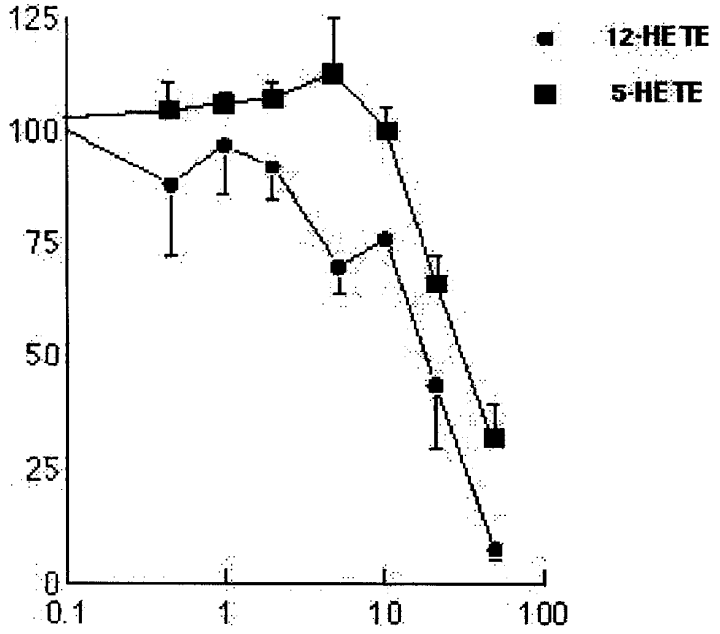
To test the ability of Lyprinol to inhibit the production of leukotrienes and 5-HETE by human neutrophils, neutrophils isolated from human blood were incubated with different concentrations of Lyprinol before being stimulated with arachidonic acid, and calcium ionophore, to activate the 5-lipoxygenase pathway. The metabolites were then extracted and quantified by the above HPLC assay. The effects of increasing concentrations of Lyprinol on neutrophil leukotriene and 5-HETE production are shown in Figures 6 and 7 respectively.

-FIGURE 6-
Inhibition by Lyprinol of the production of LTB_4 and the all-trans-isomers of LTB_4 by human neutrophils



Platelets produce 12-HETE following arachidonic acid and A23187, which can be measured using a similar protocol to the measurement of leukotrienes and 5-HETE in neutrophils. Similarly, the effects of Lyprinol on platelet 12-HETE synthesis can be measured using the same protocol as for neutrophils, as described above. Figure 7 shows the effects of increasing concentrations of Lyprinol on neutrophil 5-HETE synthesis, and platelet 12-HETE synthesis.

-FIGURE 7-
Effect of Lyprinol on 12-HETE and 5-HETE synthesis by human platelets and neutrophils respectively



In summary, Figures 6 and 7 demonstrate that short-term in vitro incubation (10 minutes) of Lyprinol with isolated human neutrophils and platelets produces a significant inhibition of leukotriene and HETE synthesis, with and IC50 (50% inhibition) of between 20 and 50 ???/ml.

Thus Lyprinol may exert its anti-inflammatory effects by inhibiting three distinctly separate stages of the inflammatory response: inhibition of chemotaxis, inhibition of transmigration of chemotactic cells through the blood vessel walls, and in the case of asthma, inhibition of the production of the broncho-constricting leukotrienes. Although other inhibitors of 5-lipoxygenase have been developed (e.g. zileuton), they have a real potential toxicity. Lyprinol is atoxic, and has very few side effects.
Furthermore, at the Lyprinol International Conference, in Nelson, New Zealand, on February 23, 2000, Michael W. Whitehouse presented his first data comparing Lyprinol vs. anti-COX2 [celecoxib, rofecoxib] commercial best sellers. Dr. Whitehouse used his mycobacterial adjuvant-induced polyarthritis rat model. Lyprinol proved **more** effective than both drugs. These results are being expanded, and will be published shortly.

Recent Clinical Studies

Arthritis

In 1998, Drs. S.L.M. and R.G. Gibson published their results on "the treatment of arthritis with a lipid extract of *Perna canaliculus*". This was a double-blind randomized 3-month study in patients with either RA [ARA criteria] or OA [clinical, radiological]. There were 60 patients enrolled for a parallel comparison study, and a further 3-month period on lipid extract for all patients. The setting was the outpatient department of the Glasgow Homeopathic Hospital, Scotland. Patients on Lyprinol received 210mg/day. The main outcome measures were: 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].

The results were impressive: 76% of RA and 70% of OA patients benefited. AI, LUT, and FI improved significantly by 3 months. One patient experienced fluid retention, and another developed transient nausea. Drs. Gibson conclude that Lyprinol is effective in reducing pain, swelling and stiffness, and in improving functional index in RA and OA.

An ongoing study is being conducted by Niels H.P. Hertz, MD, of Copenhagen, Denmark. Dr. Hertz has, so far, enrolled 13 patients with longstanding osteoarthritis in one or both knee and/or hip. The criteria for inclusion were pain, and radiological and/or arthroscopic evidence. Median duration of known OA was 4.5 years. Patients received Lyprinol: 4 capsules daily for 25 days, then 2 capsules/day. Patients were/are evaluated every 21-28 days, with VA for pain and "activity of daily life"[ADL] questionnaire. 12/13 patients reported a dramatic 50% reduction of pain [VA], and 50% improvement at day 21-28; the same results were confirmed at day 42-56. There was no increase in the use of rescue medication [pain reliever]. There were no side effects, notably no gastric pain.

Asthma

40 patients (14 males & 26 females, age 18-62), with atopic asthma were enrolled by Professor Alexander Yemelyanov, Therapeutic Clinic, Pavlov Medical University Hospital, St. Petersburg, Russia. These patients had never received corticosteroids. 30 patients received Lyprinol 2 capsules b.i.d. during 8 weeks, and 10 (being expanded to 30) received a placebo. Rescue medication was inhaled beta-2 agonist [Albuterol, Fenoterol]]. The diagnostic used the American Thoracic Society [ATS] criteria. Diagnosis was based on clinical history, and low (<15%) but reversible FEV1. The mean duration of asthma was 5.8 years; the mean FEV1 at inclusion was 86.3% of predicted. Pulmonary function tests were comprehensive. Each patient was trained to assess her/his peak flow rate b.i.d. with a Vitalograph. Eosinophil cationic protein [ECP], a severity

marker of asthma, was measured using a Pharmacia RIA. The concentration of hydrogen peroxide in exhaled air condensate was also measured.
The statistical methods used Student's paired two-tailed test, and a P value <0.05 was considered significant.
The results are very significant: Lyprinol had a significantly positive effect, already at day 28, confirmed at day 56, on:

- clinical symptoms: chest tightness, and nocturnal attacks;
- use of rescue medication [reduction of 50%];
- peak expiratory flow;
- concentration of H2O2 in exhaled air concentrate [reduction of 65%].

There was no improvement in the placebo treated group.
No side effects were reported in either group.
Professor Yemelyanov concluded: "This study has revealed some beneficial effects of Lyprinol in mild asthmatic patients. These findings provide evidence that Lyprinol may have anti-inflammatory activity on airways."

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 specific 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" (Michael W. Whitehouse).

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Dr. Georges M. Halpern is a Professor Emeritus of Medicine, and Nutrition, at the University of California, Davis, and a Visiting Professor at The University of Hong Kong. Dr. Halpern is the author of 12 books and over 300 original papers, scientific reviews and abstracts. [**Correspondence:**

Georges M. Halpern, M.D., Ph.D., D.Sc.
9 Hillbrook Drive
Portola Valley, CA 94028-7933
E-mail: ghalpern@slip.net]