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

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TITLE

Trattamento della osteoartrosi del ginocchio con Liprinol®, estratto di mollusco dalle labbra verdi – uno studio placebo-controllato in doppio cieco

KEY WORDS
Lyprinol®, chronic osteoarthritis, essential fatty acids, quality of life, natural remedies, *Perna canaliculus*

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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.

Riassunto

Il trattamento della osteoartosi (OA) prevede il controllo del dolore ed il miglioramento delle funzioni e dello stile di vita dei pazienti. Nonostante trattamenti convenzionali quali terapie a base di farmaci antinfiammatori non steroidei e semplici analgesici possano ottenere gli scopi prefissi, la loro utilizzazione non è priva di effetti collaterali. Pertanto, i pazienti affetti da OA fanno comunemente ricorso a "rimedi naturali" e a "farmaci tramandati dalla tradizione". Il Liprinol® è un estratto lipidico di mollusco dalle labbra verdi, ricco di acidi grassi omega-3, che in precedenza, attraverso studi condotti sia *in vitro* che su animali, ha dimostrato di possedere proprietà antinfiammatorie. Il presente studio ha l'obiettivo di mettere a confronto gli effetti del Liprinol® vs placebo su indicazioni, sintomi e qualità della vita del paziente, nel trattamento del ginocchio affetto da OA. Ad ottanta pazienti con OA del ginocchio, selezionati a caso, è stato somministrato Liprinol® oppure placebo per sei mesi. Nel corso dello studio, a tutti i pazienti è stata concessa la possibilità di impiegare una terapia di soccorso a base di paracetamolo e tutti i pazienti sono stati riesaminati dopo 0, 2, 4, 8, 12, 18 e 24 settimane per valutare lo stato della malattia e la sicurezza di impiego del farmaco. La valutazione dell'artrosi dei pazienti prevedeva l'uso di una scala analogica visiva di 100 mm (VAS) per il dolore, di una valutazione globale dell'artrosi ad opera del paziente e del medico, di una versione validata cinese dell'Oxford Knee Score (COKS), di una versione validata cinese della Arthritis Impact Measurement Scale 2-short form (CAIMS2-SF), della velocità di eritrosedimentazione (VES) e della proteina C reattiva (CRP). Si è osservato un miglioramento praticamente di tutti i parametri di valutazione dell'artrosi, in entrambi i gruppi di pazienti studiati. Tuttavia, il miglioramento più significativo si è registrato nella percezione del dolore misurata mediante la VAS e nella valutazione globale dell'artrosi ad opera del paziente in quei soggetti a cui era stato somministrato Liprinol® rispetto ai soggetti di controllo, a partire dalla quarta settimana dall'aggiustamento nella quantità di paracetamolo utilizzata tra le diverse visite previste dallo studio. I pazienti che avevano assunto Liprinol® ma non placebo presentavano inoltre dei punteggi migliori nella funzione fisica e nella condizione psicologica rilevate tramite CAIMS2-SF a partire dalla quarta settimana. Tuttavia, tra i due gruppi non si sono registrate differenze significative per quanto attiene a questi punteggi, in corrispondenza delle varie visite previste dallo studio. Il Liprinol®, dopo essere stato impiegato per oltre sei mesi ha mostrato di essere sicuro e di venire tollerato bene, in assenza di effetti collaterali gravi. Inoltre, non si sono registrate differenze significative tra i pazienti trattati con Liprinol® e quelli trattati con placebo per quanto attiene all'incidenza complessiva delle reazioni sgradevoli o all'abbandono dello studio, come conseguenza della tossicità del farmaco sperimentale. In conclusione, il Liprinol®, un estratto lipidico di mollusco dalle labbra verdi, può essere considerato un'opzione sicura nel trattamento della OA.

Introduction

Osteoarthritis (OA) is the most common form of chronic progressive arthritis affecting primarily elderly people. Worldwide, OA is the fifth largest contributor to disability life years (1). In the United States, OA is the leading cause of chronic disability, affecting over 20 million adults (2). In Hong Kong, OA is probably the most common form of arthritis that is associated with significant disability. In a previous random survey of the Hong Kong population aged 70 years and older, 30% reported significant symptoms of arthritis, which were more common in women (40%) than men (12%) (3). Of those with joint symptoms, 68% of women and 42% of men reported limitation in activities as a result. Further, joint pain was associated with functional impairment, depressive symptoms, increased doctor consultations, and sleep problems.

There is no cure for OA. Control of pain and improving the function and quality of life of patients are the main goals in the management of this condition. Simple analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), cyclo-oxygenase 2 (COX-2) specific inhibitors and mild opioids are recommended pharmacological agents in the treatment of OA (4). However, these drugs only provide partial relief and do not modify the course of the disease. Additionally, chronic use of these drugs is associated with significant side-effects. For example, NSAID induced upper gastrointestinal (GI) tract complications are major iatrogenic disorders. The point prevalence of upper GI ulceration has been shown to range from 10% to 20% (5). Of clinical importance are ulcers that cause symptoms or develop into potentially life-threatening ulcer complications such as upper GI bleeding, perforation, and gastric outlet obstruction. These complications are reported to occur in 2%-4% of patients taking NSAIDs for 1 year and are associated with significant morbidity and mortality (6-8). In view of the above, the use of "natural products" and "folklore medicines" is a common practice amongst patients with OA (9).

The Maoris who live in New Zealand have claimed for centuries that consuming raw local green-lipped mussels (*Perna canaliculus*) helps them maintain good health (10). Recent statistics show that the reported incidence of arthritis is extremely low in the coastal-dwelling Maoris, who consume large amounts of raw green-lipped mussels, whereas Maoris who reside in the interior have the same incidence of arthritis as New Zealanders of European origin (11). In an open label study, capsules of powdered *Perna canaliculus* were used to treat patients with various forms of arthritis. The results showed that 34.8% of patients experienced considerable improvement, 32.6% were helped to a lesser extent and 32.6% did not improve. The extract was found to be safe and well tolerated. Many patients reported that it took 3 to 4 weeks or more to notice a positive effect (12).

Lyprinol® is a patented stabilized lipid extract of *Perna canaliculus*. 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 OA of the hip and/or knee.

Patients and methods

Patients

Prior approval to conduct this study was obtained from The University of Hong Kong Medical Ethics Committee. Consecutive patients with hip or knee OA as classified according to the American College of Rheumatology Classification Criteria for at least six months were recruited from the specialist clinics of the University Departments of Medicine and Orthopaedic Surgery of the Queen Mary Hospital, a major teaching hospital in Hong Kong. Although it was originally intended to study subjects with either hip or knee OA, very few pa-

tients seen at the two clinics had hip OA. Eventually, only patients with clinical and radiographic knee OA were studied. These patients had knee pain and radiographic evidence of osteophytes and at least one of the following three features: (1) age >50 years; (2) morning stiffness <30 minutes; and (3) the presence of crepitus on physical examination (13) Patients studied were judged by the physician to require drug treatment for relief of his/her arthritis, and whose symptoms at study entry were ≥3 on a 5-point Likert global assessment scale where 1 = very well, and 5 = very poor.

The following recruitment exclusion criteria were applied: (1) co-existing inflammatory arthropathies; (2) uncontrolled co-morbidity; (3) the use of injectable or oral forms of corticosteroid within 4 weeks prior to recruitment; (4) the use of intra-articular hyaluronic acid within 6 months prior to recruitment; (5) beef allergies; and (6) dietary supplementation of omega-3 essential fatty acids such as fish oil and evening primrose oil.

Treatment and study design

This was a double-blind randomized placebo-controlled study for six months. Following a written informed consent, an eligibility screen was carried out (week -1). In addition, a plain weight bearing x-ray of the index joint was taken. Patients who fulfilled the initial clinical eligibility criteria were asked to discontinue their NSAID therapy and commence paracetamol 2 gm daily, with an additional 2 gm per day available for breakthrough pain. All were asked to record their use of paracetamol using a diary. All patients returned one week later (week 0) when baseline blood results and inclusion and exclusion criteria were reviewed. Eligible patients were randomly assigned to receive either active (Group A) or placebo (Group B) treatment for 6 months. Active treatment consisted of Lyprinol® four capsules per day (two during breakfast, two during dinner/supper) for 2 months, then two capsules per day (one capsule twice daily during meals) until the end of study. Patients in the placebo treatment group received the same number and schedule of capsules containing olive oil. All patients were instructed to adjust their paracetamol dose according to the severity of their symptoms. Reassessments were carried out at week 2, 4, 8, 12, 18 and 24. The total amount of paracetamol used between each visit was recorded. In addition, the following were performed (Table 1):

Efficacy assessment

i. Patient's assessment of arthritis pain using a 100 mm visual analog scale (VAS)

Table 1 - Study schedule

Week	-1	0	2	4	8	12	18	24
Written informed consent	X							
Eligibility screen	X	X						
X-ray of index joint	X							
Discontinuation of NSAID	X							
Randomization		X						
Efficacy assessment*	X	X	X	X	X	X	X	X
Safety assessment**			X	X	X	X	X	X
Compliance assessment***			X	X	X	X	X	X

* Efficacy assessment – Chinese Oxford Knee Score; Chinese Arthritis Impact Measurement Scales-2 short form; patient's global assessment of arthritis; physician's global assessment of arthritis; patient's 100 mm visual analog scale for pain; paracetamol use (from week 0); erythrocyte sedimentation rate; and C-reactive protein

** Safety assessment – Enquiry about drug adverse reactions since last visit; complete blood count including differential count; renal and liver function tests; clotting times (prothrombin time and activated partial prothrombin time: week –1, 12 and 24 only)

*** Compliance assessment – trial capsule count

ii Patient's global assessment of arthritis on a 5-point Likert scale
iii Physician's global assessment of arthritis on a 5-point Likert scale
iv Chinese Oxford Knee Score (COKS) (14)
v Chinese Arthritis Impact Measurement Scale 2-short form (CAIMS2-SF) (15, 16)
vi. Westergren's erythrocyte sedimentation rate (ESR)
vii. C-reactive protein (CRP) by nephelometry

Assessment of the index joint at baseline and physician's global assessment of arthritis on a 5-point Likert scale was administered by the clinical investigator. All other efficacy assessments were carried out by the study site coordinator who interviewed all patients. Since most patients were elderly and illiterate, both the COKS and CAIMS2-SF were administered with assistance from the study site coordinator. The case report forms of the study were monitored by a study monitor for data quality assurance. The study monitor visited the study site at regular intervals during the study period to ensure that the collected data were consistent and accurate. The monitor checked the recruitment progress, adherence to the study protocol and compliance to good clinical practice during each visit.

The COKS questionnaire consisted of 12 questions. Each question had five choices arranged in descending status order with 1 = the best status, and 5 = the worst status. Therefore, the score for each question ranged from 1 to 5 and the range for the COKS questionnaire was from 12 to 60, with 12 representing the best health status and 60 the worst status.

The CAIMS2-SF consisted of five subscales – physical; upper limb function; self-care; social activities; and psychological – with a total of 22 questions. For each question, there were five choices ranged in descending health status order with 0 = the best status, and 4 = the worst status. The score for each question ranged from 0 to 4 and the total score for the physical component (10 questions) was summed up to give a single score ranging from 0 to 40. The total score for the upper limb (5 questions), self-care (3 questions), social activities (1 question) and psychological (3 questions) components ranged from 4 to 20, 0 to 12, 0 to 4, and 0 to 12 respectively. The lower the score, the better the health status. Since each subscale consisted of a different number of questions, in order to be analyzed together, the scores of all the subscales were standardized with a 0 to 10 scoring system with 0 representing the best health status, and 10 the worst health status.

Safety assessment

i. Enquiry about drug adverse reactions since the last visit
ii. Whole blood count including differential counts
iii. Serum urea and creatinine
iv. Liver function tests including serum albumin, globulins, bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT)
v. Prothrombin and activated partial prothrombin times (PT and aPPT)

Compliance assessment

i. Capsule count

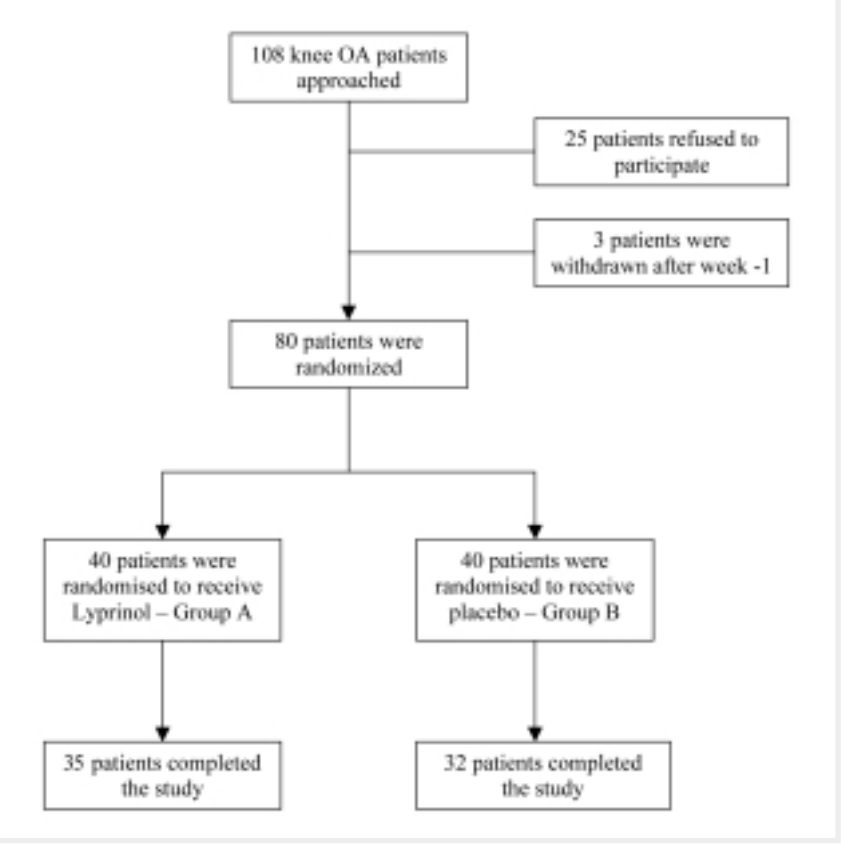
Statistical analysis

All data collected were entered into a computer database and then analysed using the statistical software Statistical Product and Service Solutions (SPSS, version 11.0 for Windows, SPSS Inc, Chicago, IL).

Descriptive statistics were used to summarize the continuous demographic and categorical data. A non-parametric test, the Mann Whitney U test, was used to compare if the two independent groups were comparable in terms of age, height, body weight and duration of OA. Another non-parametric test, the Fisher's Exact test was

used to compare if sex was significantly different between the two groups. For the analysis of efficacy and safety assessment parameters, a univariate analysis of variance for repeated measures was used with the data adjusted for the change in the amount of paracetamol used when compared with baseline (week -1) at each visit. Patient and physician global assessment data were converted into linear scales and analyzed in the same manner as other efficacy variables. Results are expressed as mean ± SD. P-values of <0.05 were regarded as being statistically significant.

Figure 1 - Patient recruitment flow chart



Results

Patients

One hundred and eight patients with knee OA were recruited. All were ethnic Chinese. Twenty-five patients declined to participate in the study. Three agreed to take part but failed the screening test and were withdrawn subsequently. Thus, a total of 80 patients agreed to participate in the study, passed the eligibility assessment and were randomized into one of the two study groups – Lyprinol® (Group A) and placebo (Group B) treatment groups (Figure 1). The demographic data of these two groups of patients are summarized in Table 2.

Efficacy assessment

The amount of paracetamol used by both groups of patients was very variable. There were no significant differences in the percentage change in paracetamol used when compared with baseline between the two groups of patients throughout the study. As the OA signs and symptoms and physical function of the patients studied could be influenced by the amount of rescue medicine used, comparison of changes in the efficacy assessment parameters between the two groups was made only after adjustment for the change in the amount of paracetamol used during the study.

The VAS for pain score was significantly reduced in both groups. In the Lyprinol® group (Group A), the mean score reduced from 63.0 at baseline to 55.5 at week 4 ($p = 0.046$), 51.2 at week 8 ($p = 0.003$),

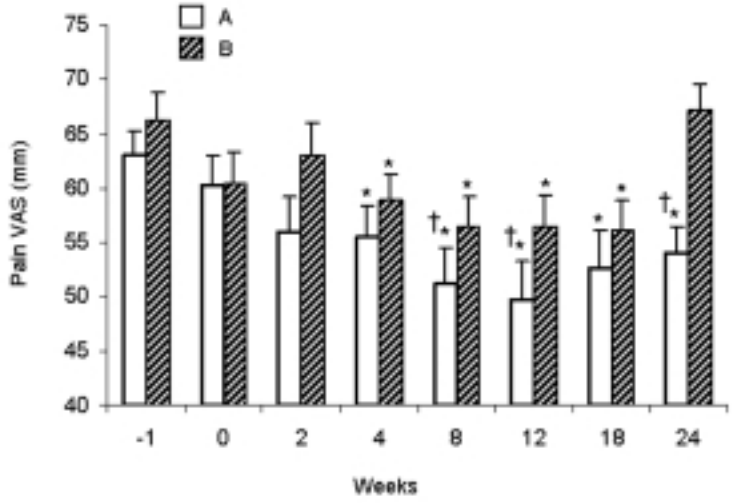
49.7 at week 12 (p = 0.001), 52.7 at week 18 (p = 0.008), and 54.0 at week 24 (p = 0.042). For patients who were treated with placebo (Group B), the mean score reduced from 66.2 at baseline to 58.8 at week 4 (p = 0.032), 56.5 at week 8 (p = 0.007), 56.5 at week 12 (p = 0.007), and 56.1 at week 18 (p = 0.003). There was a greater significant reduction in VAS pain score following adjustment for the change in the amount paracetamol used in patients who received Lyprinol® when compared with controls at week 8 (p = 0.035), week 12 (p = 0.032) and week 24 (p = 0.045) (Figure 2).

Patients' global assessment of their arthritis condition also improved in both groups during the study period. The mean score of the Lyprinol® treatment group (Group A) was reduced significantly from 3.70 to 3.08 at week 4 (p = 0.009), 2.89 at week 8 (p = 0.001), 2.95 at week 12 (p = 0.000), and 2.95 at week 18 (p = 0.000). In the placebo treatment group (Group B), the mean score was reduced from 3.75 to 3.27 at week 4 (p = 0.002), 3.24 at week 8 (p = 0.002), 3.20 at week 12 (p = 0.001), and 3.13 at week 18 (p = 0.000). There was a significantly greater reduction in the patients' global assessment score at week 12 (p = 0.035) and 18 (p = 0.04) in patients who received Lyprinol® when compared with controls following adjustment for the change in the amount of paracetamol used (Figure 3).

Table 2 - Demographic data of the two groups of patients studied. Patients in Group A were given Lyprinol® treatment while patients in Group B received placebo treatment. There were no statistical significant differences in any of these parameters between the two groups of patients studied

	Group A (n = 40)	Group B (n = 40)
Age (years), mean (range)	62.1 (47-74)	62.9 (46-80)
Male : female	5 : 35	6 : 34
Height (m), mean (range)	1.52 (1.27-1.74)	1.53 (1.26-1.79)
Weight (Kg), mean (range)	64 (44.5-93)	64.1 (44–102)
Smokers : non-smokers	2 : 38	2 : 38
Drinkers : non-drinkers	1 : 39	6 : 34
Duration of OA (months), mean (range)	128.9 (9–384)	84.7 (10–216)
Right knee : left knee : both knees	26 : 14 : 0	27 : 13 : 0

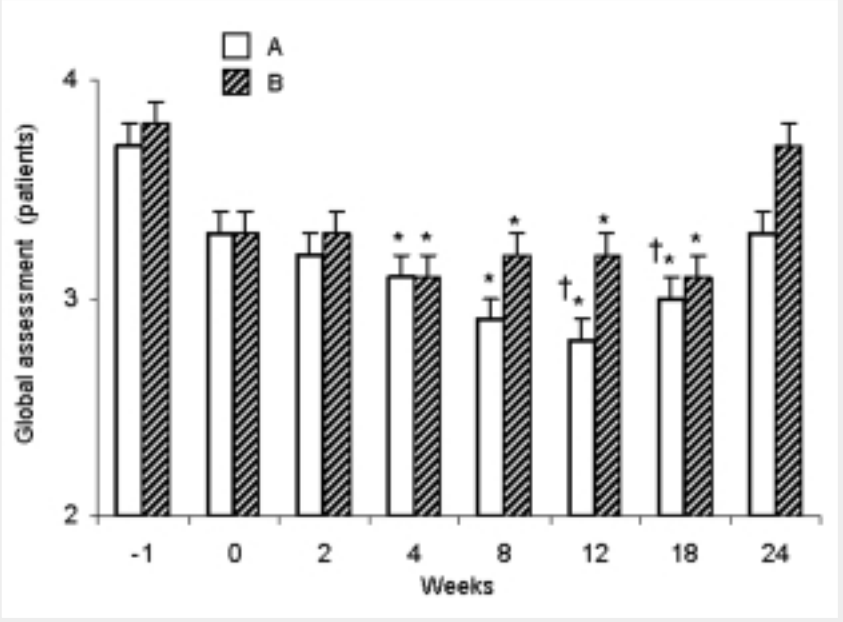
Figure 2 - Changes in the visual analog scale (VAS) for pain score of the two groups of patients studied. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean ± sem



* Significant reduction in score when compared with baseline (p<0.05)
† Significantly greater reduction when compared with Group B following adjustment for the change in the amount of paracetamol used (p<0.05)

Improvement in other efficacy assessment parameters including the

Figure 3 - Changes in patients' global assessment of their arthritis score of the two groups of patients studied. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean ± sem



physician's global assessment score and COKS was also noted but no significant differences were detected between the two groups during the study (Table 3). The physical and psychological domain scores of the CAIMS2-SF were reduced significantly at week 4, 8 and 12, and week 8 and 12, respectively in Lyprinol® treated patients. No significant changes in the physical and psychological CAIMS2-SF domain scores were noted in the placebo group (Figures 4 and 5). However, there were no significant differences in these scores between the two groups of patients during the study period. The upper limb function, self-care and social domain scores of the CAIMS2-SF did not significantly change during the study in either group of patients (Table 4).

Withdrawal and adverse reactions

Five and eight patients withdrew from the Lyprinol® (A) and placebo (B) groups respectively for various reasons (Table 5). Two patients from Group B withdrew from the study but no specific reasons were given. One patient who received Lyprinol® and three patients who took placebo withdrew due to lack of efficacy of the trial medication. One patient from group A developed multiple joint pain during the study period. Clinical assessment showed active synovitis involving multiple metacarpophalangeal and proximal inter-phalangeal joints. Her serum rheumatoid factor was later found to be positive. A diagnosis of rheumatoid arthritis (RA) was made and the patient was withdrawn from the study. Another patient who was on placebo treatment developed increased pain in her knee. She visited her family physician and was given an intra-articular injection of steroid. She was subsequently withdrawn from the study. Adverse events occurred in four patients – three in Group A and one in Group B. One patient from Group A complained of nausea. Although it was not severe, the patient elected to discontinue from the study. Two patients, in each treatment group, developed elevated serum liver aminotransferase levels to ≥2 upper range of normal and were withdrawn from the study. Both patients had further investigations to elucidate the cause of their liver enzyme derangement. No causes were found. The aminotransferase levels remained static and persistently elevated. Since both patients were well, no treatment was given. The abnormal liver enzyme levels were not thought to be related to the trial medication. One patient from Group A developed heart failure. This, however,

Table 3 - Changes in efficacy assessment parameters of the two groups of patients studied. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean (standard deviation).

	Group	Week							
		-1	0	2	4	8	12	18	24
% charge in paracetamol used	A		100	48.9 (196.4)	54.8 (152.1)	127.5 (245.9)	162.0 (322.8)	237.8 (472.4)	272.7 (525.2)
	B		100	92.6 (402.4)	92.7 (422.4)	208.0 (533.7)	128.6 (332.0)	162.7 (300.2)	189.2 (304.1)
Pain VAS (mm)	A	63.0 (14.3)	60.3 (16.6)	56.0 (18.8)	55.5 (16.9)*	51.2 (20.6)*†	49.7 (20.1)*†	52.7 (21.2)*	54.0 (15.2)*†
	B	66.2 (15.7)	60.4 (18.2)	63.0 (18.0)	58.8 (14.1)*	56.5 (15.8)*	56.5 (16.3)*	56.1 (17.2)*	67.1 (5.5)
Global (patients)	A	3.7 (0.7)	3.3 (0.6)	3.2 (0.7)	3.1 (0.7)*	2.9 (0.6)*	2.8 (0.8) *†	3.0 (0.8) *†	3.3 (0.7)
	B	3.8 (0.7)	3.3 (0.6)	3.3 (0.7)	3.1 (0.6)*	3.2 (0.6)*	3.2 (0.7)*	3.1 (0.7)*	3.7 (0.7)
Global (physician)	A	3.4 (0.6)	3.1 (0.6)	2.8 (0.6)	2.9 (0.6)*	2.8 (0.7)*	2.8 (0.7)*	2.8 (0.8)*	3.5 (0.6)
	B	3.3 (0.5)	3.1 (0.7)	3.1 (0.7)	2.9 (0.7)*	2.9 (0.7)*	2.9 (0.7)*	2.9 (0.6)*	3.3 (0.5)
COKS	A	20.3 (6.5)	19.0 (5.2)	16.3 (4.9)	15.7 (5.6)*	14.7 (5.3)*	14.9 (5.5)*	16.1 (7.4)*	18.1 (6.5)
	B	19.5 (6.0)	18.0 (5.0)	17.4 (4.7)	16.5 (5.8)*	17.0 (5.5)	16.6 (5.9)*	16.1 (5.3)*	19.2 (5.6)
ESR ($mm\ hr^{-1}$)	A	19.3 (11.2)		21.4 (13.0)	21.5 (13.3)	20.7 (14.4)	22.3 (15.1)	21.8 (13.6)	20.5 (13.2)
	B	30.1 (23.1)		28.5 (21.4)	28.2 (22.3)	28.1 (23.4)	27.4 (22.7)	26.0 (20.5)	26.4 (20.1)
CRP (mg/dl)	A	0.5 (0.4)		0.4 (0.3)	0.5 (0.5)	0.5 (0.4)	0.5 (0.6)	0.4 (0.1)	0.4 (0.2)
	B	0.5 (0.4)		0.6 (0.5)	0.5 (0.4)	0.5 (0.3)	0.6 (0.8)	0.5 (0.3)	0.5 (0.4)

VAS = visual analog scale; COKS = Chinese Oxford Knee Score; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein

* Significant reduction in score when compared with baseline ($p<0.05$)

† Significantly greater reduction when compared with Group B following adjustment for the change in the amount of paracetamol used ($p<0.05$)

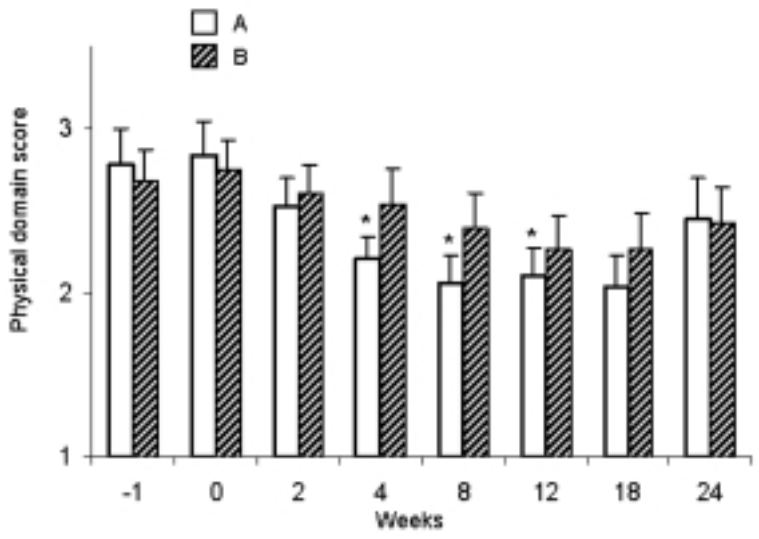
was not considered by the investigator to be associated with the trial medication. No other adverse reactions were noted in either group of patients during the study. One patient from Group B was discontinued from the study due to poor compliance.

There were no significant changes in the blood parameters – whole blood count and differential counts, renal and liver function tests, and clotting times – of the two groups of patients studied during the study. Similarly, blood pressure, other than that measured at week -1 when it was found to be higher, did not vary significantly throughout the study (Table 6).

Discussion

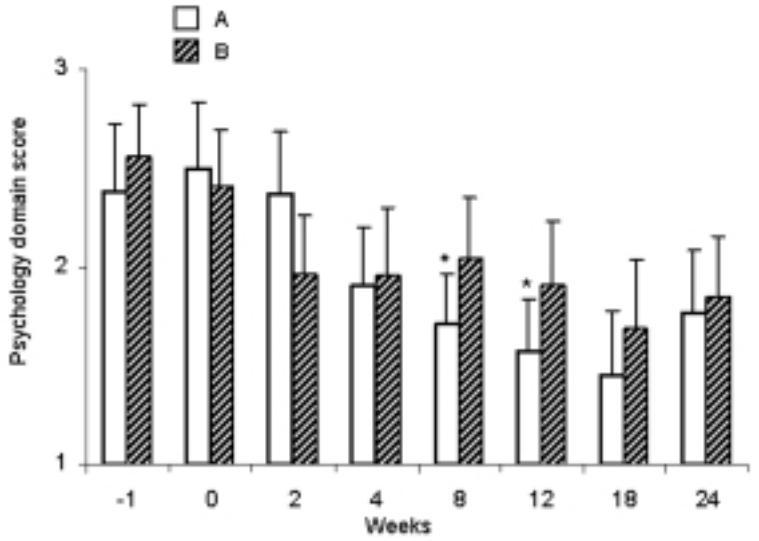
This is the first randomized controlled trial that evaluates the effects of Lyprinol®, a lipid extract of the green lipped mussel (*Perna canaliculus*), on the signs and symptoms and quality of life of patients with knee OA. Our results show that the use of Lyprinol® over 6 months in pa-

Figure 4 - Changes in the physical domain score of the Chinese Arthritis Impact Measurement Scale 2 – Short Form of the two groups of patients studied. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean ± sem



* Significant reduction in score when compared with baseline ($p<0.05$)

Figure 5 - Changes in the psychological domain score of the Chinese Arthritis Impact Measurement Scale 2 – Short Form of the two groups of patients studied. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean ± sem



* Significant reduction in score when compared with baseline ($p<0.05$)

tients with knee OA is well tolerated and associated with a decrease in pain perception and patient's global assessment of his/her arthritis status after two months when compared to placebo. In addition, there was a tendency to improvement in the majority of other efficacy assessment parameters in patients who were given Lyprinol® when compared with those treated with placebo, although the differences did not reach statistical significance.

The potential use of powdered *Perna canaliculus* in patients with arthritis was first explored by Gibson et al (12). The extract was found to be safe and well tolerated. The chemical nature and structure of the anti-inflammatory constituents of this agent were later characterized using both gas chromatography and mass spectrometry (17). This was found to be a lipid fraction, identified as Lyprinol®, which contains a unique combination of triglycerides, sterol esters, free fatty acids, polar lipids and carotenoids. Subsequent studies showed that Lyprinol® exhibits its anti-inflammatory effects through inhibition of the synthesis of inflammatory leukotrienes (LTs) and possibly some prostaglandins (PGs), 5-lipoxygenase and cycloxygenase metabolites respectively of arachidonic acid, the most abundant form of essential fatty acid in our body (11, 18).

In 2000, Sinclair et al (19) reported a comparison between the composi-

Table 4 - Changes in the score of five domains of the Chinese Arthritis Impact Measurement Scale-2 Short Form of the two groups of patients studied. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean (standard deviation).

	Group	Week							
		-1	0	2	4	8	12	18	24
Physical	A	2.78 (1.34)	2.84 (1.27)	2.53 (1.11)	2.20 (0.85)*	2.05 (1.11)*	2.10 (1.04)*	2.03 (1.17)	2.44 (1.63)
	B	2.68 (1.17)	2.74 (1.18)	2.60 (1.13)	2.54 (1.28)	2.39 (1.29)	2.26 (1.25)	2.26 (1.26)	2.43 (1.39)
Upper	A	0.45 (1.30)	0.63 (1.40)	0.69 (1.41)	0.55 (1.64)	0.43 (1.04)	0.33 (1.04)	0.28 (0.89)	0.49 (1.09)
limb	B	0.59 (1.35)	0.74 (1.74)	0.88 (1.92)	0.73 (1.76)	0.81 (1.95)	0.74 (1.82)	0.56 (1.05)	0.65 (1.50)
Selfcare	A	0.04 (0.26)	0.04 (0.26)	0.09 (0.53)	0.27 (1.64)	0.67 (0.30)	0.00 (0.00)	0.02 (0.14)	0.06 (0.40)
	B	0.15 (0.80)	0.15 (0.80)	0.15 (0.67)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.08 (0.13)
Social	A	5.56 (2.23)	5.69 (2.19)	5.45 (2.56)	5.74 (2.11)	5.41 (2.00)	5.07 (2.20)	5.74 (2.26)	6.09 (1.97)
	B	5.56 (2.23)	5.44 (2.26)	5.40 (2.43)	5.54 (2.30)	5.71 (2.23)	5.81 (2.66)	5.70 (2.40	5.94 (2.17)
Psychology	A	2.38 (2.12)	2.50 (2.06)	2.37 (1.96)	1.91 (1.76)	1.71 (1.60)*	1.57 (1.64)*	1.45 (1.94)	1.77 (1.94)
	B	2.56 (1.62)	2.42 (1.78)	1.97 (1.81)	1.96 (2.07)	2.05 (1.82)	1.91 (1.89)	1.69 (1.90)	1.85 (1.91)

* Significant reduction in score when compared with baseline ($p<0.05$)

tion of the oil derived from Lyprinol® and two other oils rich in omega-3 fatty acids, namely flaxseed oil and tuna oil. The main lipid classes in Lyprinol® are sterol esters, triglycerides, free fatty acids, sterols and phospholipids while triglycerides are the main lipids in the other two oils. The main omega-3 fatty acids in Lyprinol® are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while α-linolenic acid (ALA) and DHA are the main omega-3 acids found in the flaxseed oil and tuna oil. The main sterols in Lyprinol® are cholesterol and desmosterol/brassicasterol, while in flaxseed oil and tuna oil the main sterols are beta-sitosterol and cholesterol, respectively. Epidemiological observations, population studies and basic research indicate the possibility of influencing the outcome of cardiovascular disease, inflammatory disorders, neural function by ingestion of the omega-3 polyunsaturated fatty acids (19, 20).

Table 5 - Summary of patients withdrawn from the two groups during the study period. Group A = Lyprinol® treatment; Group B = placebo treatment

Reasons for withdrawal from study	Group A	Group B
Patient did not want to continue study	0	2
Lack of efficacy	1	3
Development of exclusion criteria	1 (RA)	1 (IA steroid)
Adverse event		
- nausea	1	
- abnormal liver enzymes	1	1
- heart failure	1	
Poor compliance		1
Total	5	8

RA = rheumatoid arthritis; IA = intra-articular

Table 6 - Changes in safety assessment parameters of the two groups of patients studied. There were no significant changes in any of these parameters both within and between the two groups during the study period. Group A = Lyprinol® treatment; Group B = placebo treatment. Results are expressed as mean (standard deviation).

	Group	Week							
		-1	0	2	4	8	12	18	24
WBC	A	4.8 (1.1)		4.9 (1.1)	5.1 (1.4)	5.0 (1.3)	5.2 (1.2)	5.2 (1.4)	5.4 (1.4)
($x10^9$/l)	B	4.9 (1.0)		4.8 (1.1)	4.7 (1.1)	4.9 (1.0)	4.8 (1.0)	4.9 (1.1)	5.0 (1.1)
Hgb	A	13.2 (1.1)		13.1 (1.2)	13.0 (1.2)	12.9 (1.1)	12.9 (1.3)	13.1 (1.2)	13.1 (1.2)
(g/dl)	B	13.1 (1.5)		12.9 (1.3)	12.8 (1.3)	12.8 (1.4)	12.9 (1.4)	13.0 (1.4)	13.0 (1.5)
Plt	A	261 (62)		267 (58)	265 (59)	264 (65)	262 (70)	264 (61)	269 (63)
($x10^9$/l)	B	249 (60)		250 (50)	245 (54)	249 (44)	252 (48)	259 (54)	258 (50)
PT	A	11.4 (0.6)					11.2 (1.6)		11.6 (0.6)
(sec)	B	11.4 (0.4)					11.4 (0.4)		11.4 (0.6)
aPPT	A	27.9 (2.4)					27.8 (2.6)		27.2 (2.8)
(sec)	B	28.2 (2.9)					28.0 (2.6)		27.3 (2.9)
Alb	A	43.2 (2.6)		42.5 (2.1)	42.3 (2.1)	42.4 (1.7)	42.9 (2.5)	42.7 (1.7)	42.5 (1.9)
(g/l)	B	42.7 (2.1)		42.0 (2.7)	42.0 (3.2)	42.0 (3.0)	42.4 (3.0)	42.5 (2.3)	41.9 (2.8)
ALP	A	83.7 (22.2)		84.1 (23.2)	86.6 (25.1)	86.3 (23.8)	86.9 (26.5)	84.4 (21.7)	85.0 (25.6)
(u/l)	B	88.5 (25.4)		87.7 (29.2)	88.1 (29.4)	83.6 (19.7)	85.5 (23.4)	84.9 (23.6)	89.9 (27.9)
ALT	A	21.5 (8.7)		22.8 (9.0)	28.1 (35.3)	23.4 (9.1)	29.6 (22.3)	33.2 (42.3)	31.6 (50.0)
(u/l)	B	24.3 (13.4)		21.6 (9.9)	21.6 (9.9)	20.5 (7.7)	22.1 (10.6)	25.3 (12.5)	24.3 (13.9)
AST	A	22.6 (10.4)		21.5 (4.6)	23.6 (16.0)	22.1 (4.3)	25.1 (11.5)	29.7 (25.7)	26.1 (21.5)
(u/l)	B	23.0 (7.2)		22.3 (6.1)	22.3 (6.0)	21.7 (5.2)	22.9 (6.7)	24.1 (6.8)	24.7 (8.9)
BP sys	A	148.3 (26.4)	146.0 (23.2)	138.2 (25.0)	132.6 (25.7)	138.0 (26.4)	141.4 (28.5)	138.2 (22.5)	136.3 (32.5)
(mmHg)	B	144.4 (23.1)	134.0 (19.3)	130.1 (32.0)	130.7 (17.3)	131.2 (20.4)	131.7 (29.1)	139.0 (24.8)	136.6 (23.7)
BP dias	A	78.7 (10.1)	72.7 (10.4)	72.0 (11.6)	72.6 (11.6)	72.2 (11.4)	73.9 (11.3)	73.7 (10.2)	72.4 (10.3)
(mmHg)	B	78.2 (12.2)	72.3 (10.5)	73.5 (13.4)	72.5 (12.2)	71.6 (11.7)	74.4 (10.3)	74.1 (11.9)	73.0 (12.7)

WBC = white blood cell count; Hgb = haemoglobin; plt = platelet count; PT = prothrombin time; aPPT = activated partial prothrombin time; Alb = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BP sys = systolic blood pressure; BP dias = diastolic blood pressure

The parent fatty acid in the omega-3 fatty acid family is ALA which is a major fatty acid found in high concentrations in certain plant oils, such as flaxseed oil, walnut oil and canola oil. Several longer chain or derived omega-3 fatty acids are formed from ALA and these are mainly found in fish, fish oils and other marine organisms. The main marine omega-3 fatty acids are EPA, docosapentaenoic acid and DHA. It is of interest that DHA is specifically localized in the retina and the brain in humans and other mammals. The longer chain omega-3 fatty acids are rapidly incorporated into cell membrane phospholipids where they are believed to influence the metabolism/metabolic events within the cell. The mechanisms by which these changes occur include alteration in the fluidity of membranes such that there are subtle changes in receptor function, alteration in cell signalling mechanisms, membrane bound enzymes, regulation of the synthesis of eicosanoids, and regulation of gene expression.

In 1997, Whitehouse (cited by Halpern (21)) also conducted a study on Lyprinol® and showed it had significant anti-inflammatory activity when given to animals and tested with human platelets. When treated once a day with Lyprinol®, Wistar and Dark Agouti rats developed neither adjuvant-induced polyarthritis nor collagen II-induced auto-allergic arthritis. This was achieved with doses less than commonly used NSAIDs, and 200 times less than other seed or fish oils. Lyprinol® subfractions inhibited LTB4 biosynthesis by polymorphonuclear cells *in vitro* and PGE2 production by activated macrophages. Much of this anti-inflammatory activity was associated with omega-3 polyunsaturated fatty acids and natural anti-oxidants e.g. carotenoids. In contrast to NSAIDs, Lyprinol® was non-toxic to the stomach in disease-stressed rats at 300 mg/kg once daily, and did not affect platelet aggregation of the human and rat.

Besides arthritis, other studies have demonstrated that Lyprinol® has significant anti-inflammatory activity in other related conditions (22, 23). Recently, a double-blind clinical trial on 60 patients with chronic arthritis was conducted at the West Glasgow Hospital University NHS Trust (24). There were 30 RA and 30 OA patients. Both groups of patients showed significant improvement with 76.7% of the RA and 70% of the OA patients benefiting from the trial.

In our study, we had originally intended to study patients with significant OA involvement of either the hip or knee. However, only patients with knee OA were recruited and studied. This was in accordance with observations made from previous epidemiological studies. For example, in 1973, Hoaglund et al (25) observed that the prevalence of OA of the hip was 1% or lower in adult Chinese, and the prevalence of OA of the knee was 13% in elderly Chinese women and 5% in elderly Chinese men. These findings were confirmed in a subsequent study that showed that the rate of hip joint replacement in Chinese Americans was 10% of the one found in American Caucasians (26). In a local study carried out by Lau et al (27), hip OA was also found to be uncommon in ethnic Chinese. The ethnic difference in the prevalence of OA of the knee and hip may be attributable to both genetic and lifestyle factors.

Results of our study underline the importance of applying a randomized double-blind placebo controlled design when testing experimental therapies in a clinical setting. This is particularly relevant when testing natural therapies where great placebo effects are expected (28). Improvement in almost all of the efficacy assessment parameters was observed in both groups of patients studied. However, there was a greater improvement in the perception of pain as measured by a VAS, and patients' own assessment of their arthritis condition in patients who took Lyprinol® when compared with controls. Using the validated CAIMS2-SF, we were also able to show a significant improvement in the physical function and psycho-

logical status of patients who were treated with Lyprinol® but not with placebo, although there were no differences in the change of these scores between the two groups at various study visits. Besides, there was a tendency for a greater improvement in the assessment of the patients' arthritis using the physician global and the COKS scores in patients who were treated with Lyprinol® when compared with controls. It should, however, be noted that differences in these scores did not reach statistical significance.

The beneficial effects of Lyprinol® became apparent four weeks after commencement of treatment. This is in accordance with what has been reported previously (12 ,24). This delayed effect is similar to the one observed in the dietary supplementation of other forms of essential fatty acids in the treatment of arthritis (20, 29).

It is often a misconception of most patients that natural or traditional treatment remedies are free of side-effects. In truth, however, many of these agents are associated with various adverse reactions, some of which may be life-threatening (30). The other major reason for conducting controlled clinical trials on natural remedies is, therefore, to evaluate the safety profile of these agents. In our study, we have shown Lyprinol® to be well tolerated. 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. Similar observations were made in both animal and human studies that involved Lyprinol®. Of particular importance is that there were no reports of upper GI toxicity. This may be an advantage over NSAIDs, drugs that are commonly used in the treatment of OA. It should be noted, however, that the number of patients tested in this study was small for a proper evaluation of drug-related upper GI toxicity outcome. Further, no direct comparison between Lyprinol® and NSAIDs was made in this study. This should be considered in future studies.

In conclusion, we have carried out a six-month double-blind placebo-controlled study on Lyprinol®, a lipid extract of the green-lipped mussel, in the treatment of knee OA. Lyprinol® was shown to be safe and well tolerated. In addition, there was a greater reduction in the level of pain in Lyprinol®-treated patients who also reported a greater improvement in the global assessment of their arthritis condition when compared with those who were treated with placebo. Lyprinol® may be considered as another, safe option in the treatment of OA.

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