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A phase I study to determine the safety, tolerability and maximum tolerated dose of green-lipped mussel (*Perna canaliculus*) lipid extract, in patients with advanced prostate and breast cancer

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Background: This was a phase I trial to determine the maximum tolerated dose (MTD) of a marine lipid extract from the New Zealand green-lipped mussel (*Perna canaliculus*), as an inhibitor of 5- and 12-lipo-oxygenase enzymes, in patients with advanced breast and prostate cancers.

Patients and methods: This was an open-labelled, phase I, dose-escalation study. Proprietary form of green-lipped mussel lipid extract (GLMLE), 260-mg capsule, was administered on a twice-daily schedule, orally. Patients remained on study until disease progression or unacceptable toxicity.

Results: From December 1999 to May 2003, 17 patients were enrolled. Fifteen of them were male with advanced prostate cancer and two were female with advanced breast cancer. The median age of the patients was 74 years (range 56–85 years). Sixteen patients were assessable for adverse events and dose-limiting toxicity (DLT). Reason for withdrawal from the study included progressive disease (n = 12), death (n = 1) and DLT (n = 3). Two patients had evidence of grade 4 hepatic dysfunction. The MTD was not reached. There were no objective tumour responses noted.

Conclusions: GLMLE appears to be a well-tolerated compound in this setting. There appears to be no objective benefit. However, grade 3/4 hepatic toxicity noted in two patients is of concern and should be considered while evaluating patients taking GLMLE or while designing studies with this agent.

Key words: breast cancer, green-lipped mussel, Lyprinol, prostate cancer

introduction

The lipo-oxygenase (LOX) pathways, one of three major pathways of arachidonic acid metabolism, form hydroxyeicosatetraenoic acids (HETEs) and leukotrienes. The biological properties of the leukotrienes have been well characterised, and the biological properties of the HETEs have been shown to play an important role in tumour cell proliferation or growth (5-HETE) and metastases (12-HETE). They also have been shown to be mitogenic, to stimulate the expression of oncogenes and to be important in the inhibition of apoptosis. Tumour cell LOX activity can be activated by a variety of growth factors (e.g. epidermal and platelet-derived growth factors) and cytokines (e.g. interleukins 1, 4 and 8).

Prostate and breast cancers have featured strongly in research linking LOX metabolites and cancer. It has been shown that 12 LOX expression is high in prostate cancer tissues of patients and that degree of expression correlated with advanced stage, poor differentiation and poor prognosis [1, 2]. Correlation between increased 5-HETE production and cell growth in the androgen-insensitive prostate cancer cell line PC-3 has been demonstrated. Tumour cell growth has been shown to be strongly inhibited by MK886, a specific 5-LOX inhibitor, and this inhibition was prevented by the co-addition of 5-HETE. The same effect of HETEs in androgen-dependent (LNCaP) prostate cancer cells has also been demonstrated [3–11].

LOX-transfected PC-3 cells injected into nude mice have been shown to decrease necrosis and increase vascularisation of the tumours via increased vascular endothelial growth factor production [12]. These preclinical data provided a strong argument for the use of LOX inhibitors in the treatment of cancer, in particular prostate cancer.

GLMLE (Lyprinol®; Pharmalink Marketing Services Pty. Ltd, Burleigh Heads, Queensland, Australia) is a marine lipid extract from the New Zealand green-lipped mussel (*Perna canaliculus*) and is obtained by supercritical extraction of the

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total lipids from the stabilised, freeze-dried mussel flesh. It contains a number of lipid classes including sterol esters, triglycerides, free fatty acids (saturated and unsaturated), sterols and polar lipids. Lyprinol® has been shown to be a potent inhibitor of the LOX pathways, in particular, the 5- and 12-LOX enzymes [13] and has been found to induce apoptosis in cultured PC-3 cell lines [14]. Since it was launched in the world market in 1998 as an anti-inflammatory agent, Lyprinol® has been studied in a number of clinical trials for its effects in osteoarthritis and rheumatoid arthritis and asthma. A recent systematic review of Lyprinol® in the therapy of arthritis indicated that results thus far are inconsistent and indicated further investigation [15]. In a trial of 36 patients with atopic asthma, Lyprinol® was seen to decrease daytime wheeze and increase morning peak expiratory flow in a randomised placebo-controlled clinical trial [16]. At the time of this clinical trial, the use of LOX inhibitors as anticancer agents in clinical studies had been limited. Despite the lack of clinical evidence, there was significant media interest based on preclinical results that led to significant patient use (British Broadcasting Corporation) [17]. Based on the publicity, the American Cancer Society continues an information page on Lyprinol® (http://www.cancer.org/docroot/ETO/content/ETO_5_3X_Lyprinol.asp).

This study was a phase I, open-labelled, dose-escalation trial of Lyprinol® in treatment-refractory breast and prostate cancers. The primary objective was to determine the safety, tolerability and maximum tolerated dose (MTD) of Lyprinol® when administered on a twice-daily (b.i.d.) oral dosing schedule. The secondary objective was to evaluate response.

patients and methods

eligibility criteria

Patients were eligible for this study if they were >18 years old, had an Eastern Cooperative Oncology Group performance score of two or less and had a biopsy-proven diagnosis of advanced chemorefractory and hormone-refractory breast or hormone-refractory prostate cancer, for whom no standard therapies were available. They needed to have measurable disease with a life expectancy of >3 months. All patients were required to give written informed consent. Additionally, all patients were required to comply with the treatment protocol and be able to swallow the medication capsules. The Queen Elizabeth Hospital Ethics Committee approved the trial.

treatment

Lyprinol® capsules (260 mg) were administered, on an outpatient basis, orally using a b.i.d. dosing schedule, with medication taken not less than 10 h apart.Patients were evaluated for tumour response using appropriate diagnostic imaging and biochemical parameters at baseline and every 2 months subsequently. Patients were removed from study for documented disease progression or unacceptable toxicity.

dose escalation

Six dose levels were administered, ranging from three capsules b.i.d. to eight capsules b.i.d. (Table 1). Patients were entered in cohorts of three, starting at dose level 1. Minimum of 1 month of treatment was administered in each cohort before the next cohort was recruited.

Toxic effects were graded according to the National Cancer Institute—Common Terminology Criteria for Adverse Events (version 3).

Table 1. Patient demographics and clinical characteristics

Characteristic	No. of patients
Sex	
Male	15
Female	2
WHO performance status	
0	4
1	8
2	4
Not available	1
Diagnosis	
Breast adenocarcinoma	2
Prostate adenocarcinoma	15
Prior treatment	
Surgery	8
Radiotherapy	12
Chemotherapy	2
Hormonal therapy	14

WHO, World Health Organization.

A dose-limiting toxicity (DLT) was defined as any haematologic or non-haematologic toxicity greater than or equal to grade 3 or any toxicity requiring dose cessation. If a DLT was observed in two or more patients within the same cohort at a particular dose level, within the first 3 months of treatment, the previous dose level was to be considered the MTD and all patients on study and subsequent patients will use that dose for the remainder of the study.

If a DLT was observed in only one patient within a cohort at a particular dose level, within the first 3 months of study, the next five patients entered into the study will commence treatment at that dose level. If another DLT was observed in any additional patient tested at this dose level, the previous dose level will be considered the MTD and all further patients on study would be treated at MTD. If no further grade 3 or greater toxicity is observed in the extra five patients tested at that dose level, the next dose level will be tested as previously outlined.

pretreatment and follow-up studies

Pretreatment studies were done within 7 days before the start of medication. These included full history and clinical examination, baseline tumour measurement, complete blood examination, biochemistry, whole-body bone scan, lipid profile and tumour markers [CA 15-3, carcinoembryonic antigen for breast cancer and prostate-specific antigen (PSA) for prostate cancer]. Biochemistry and haematology were repeated a week later and if normal were then done on a monthly basis. Lipid profile was repeated every 1–2 months and tumour markers were evaluated every 2–4 weeks. Imaging and measurements were repeated bimonthly.

Tumour response based on radiologic evidence was evaluated using the World Health Organization criteria. Based on tumour marker levels, response was defined as reduction in baseline tumour marker level, of at least 50%, that was maintained for a minimum of 3 weeks, while progression was defined as an increase in tumour marker level of at least 25% from nadir in patients with no response or 50% in others, over at least 3 weeks. Stable disease was defined as <25% increase in tumour marker level from baseline, maintained for at least 3 weeks. Duration of tumour response was defined as the time between first and last evaluation, at which response criteria were met. Pain scores were evaluated using an unvalidated questionnaire. Responses were scored on a scale from 3 to 12, with the lower score indicating no pain and the higher score indicating maximum pain.

Table 2. The dose levels, number of patients treated at each level, cumulative dose administered and number of patients assessable for DLTs

Dose level	Dose (mg/day)	No. of patients entered	No. of days on treatment	No. of patients requiring dose reduction	Cumulative dose received (g)	No. of patients assessable for DLTs
1	1560	3	214	0	333.8	3
2	2080	4	229	0	476.3	4
3	2600	3	115	0	299.0	2
4	3120	3	306	0	954.7	3
5	3640	2	76	0	276.6	2
6	4160	2	105	0	436.8	2

DLTs, dose-limiting toxic effects.

statistical analysis

Summary descriptive statistics of patient demographics and other baseline features were generated for each dose level and the entire patient cohort. All patients who received at least one dose of the drug were analysed for toxicity. Laboratory assessment was summarised at baseline and significant changes were noted. The MTD was to be expanded to six patients for prostate cancer if there was a reduction in PSA (≥50%) in at least 1 of 12 patients.

results

patients

From December 1999 to May 2003, 17 patients were enrolled. Fifteen of them were male with advanced prostate cancer and two were female with advanced breast cancer. The median age of the patients was 74 years (range 56–85 years). Patient demographics, clinical characteristics and previous treatment details are listed in Table 1. Sixteen of the 17 patients received at least one dose of treatment and were assessable. One patient had evidence of disease progression and subsequently died before the commencement of trial medication. The dose levels, number of patients treated at each level, cumulative dose administered and number of patients assessable for DLTs are listed in Table 2. Patients were enrolled sequentially from dose level 1 to 6. The MTD was not reached up to cohort 6. The PSA levels for 14 patients are shown in Figure 1.

toxicity

Sixteen patients were assessable for adverse events and DLTs. Reasons for withdrawal from the study included progressive disease ($n = 12$), death ($n = 1$) and DLT ($n = 3$). Nineteen adverse events were noted among the entire cohort (Table 3). Three patients experienced an adverse event greater than or equal to grade 3. Two of them had evidence of liver function abnormalities (Table 4), while one had evidence of grade 3 anaemia. The most common adverse event was dyspepsia and anaemia ($n = 4$).

The DLTs included two patients with grade 4 hepatic dysfunction and one patient with grade 2 dyspepsia. One of the patients with hepatic dysfunction had extensive liver involvement with metastasis while the other did not have any radiological evidence of liver involvement with cancer. There were no significant alterations in the serial measures of serum triglycerides and cholesterol levels. None of the patients from the entire cohort required doses of their medication to be modified.

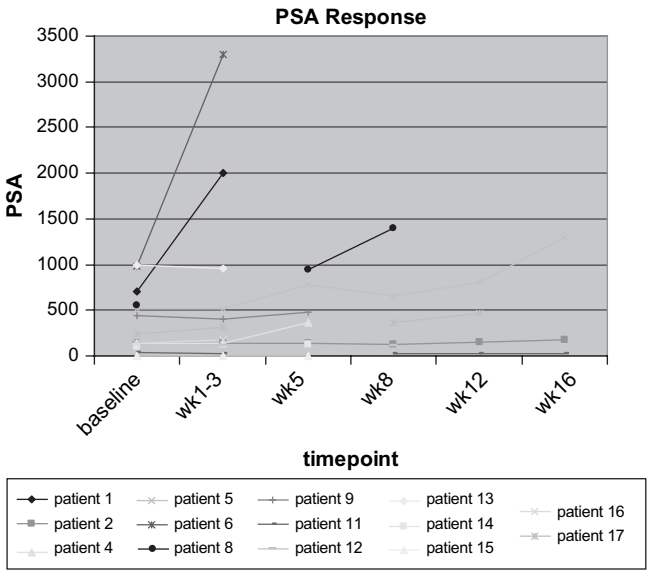

Figure 1. Plot of prostate-specific antigen (PSA) levels (*y*-axis) versus time point in weeks (*x*-axis) for 14 patients with prostate cancer.

tumour response

None of the patients had an objective radiological response. The PSA levels of patients with prostate cancer are depicted in Figure 1. Based on tumour marker levels, 11 patients had evidence of progressive disease. PSA remained stable for five patients, ranging from 3 to 12 weeks (median 5 weeks), before progressing. In three of these patients the radiological findings remained stable, while in two patients radiological correlation was not available. In the five patients with stable PSA, the pain scores similarly remained stable. No patient has a reduction in PSA level or reduction in pain score. As there was no PSA response in 15 patients, the final cohort was not expanded. The median survival for the prostate group was 124 days. All the patients in the breast cancer cohort progressed.

discussion

This phase I dose-escalation trial determined the safety profile and response of refractory prostate and breast cancer patients to Lyprinol®. MTD levels were not reached up to cohort 6, indicating that this is probably a safe agent in this setting.

Table 3. Incidence of Lyprinol®-related adverse events

Adverse event	Lyprinol® dose cohort						
	780 mg b.i.d. ($n = 3$)	1040 mg b.i.d. ($n = 4$)	1300 mg b.i.d. ($n = 2$)	1560 mg b.i.d. ($n = 3$)	1820 mg b.i.d. ($n = 2$)	2080 mg b.i.d. ($n = 2$)	All patients ($n = 16$)
Diarrhoea	0	0	1	0	0	0	1
Dyspepsia	1	0	1	2	0	0	4
Hepatic dysfunction	0	(1)	(1)	0	0	0	2
Constipation	0	1	0	1	1	0	3
Appetite loss	0	0	0	2	0	0	2
Anaemia	1	2 (1)	0	1	0	0	4
Nausea/vomiting	0	0	1	0	0	0	1
Lethargy	0	0	0	1	0	0	1
Abdominal pain	0	1	0	0	0	0	1
Total							19

Cells depict total number of patients who experienced any toxicity, within each cohort; numbers in parentheses denote patients with grade 3/4 toxicity. b.i.d., twice-daily dosing.

Table 4. Grade 3/4 liver function abnormalities in two patients

	Baseline bilirubin ($N = 6$–24 mmol/l)	Peak bilirubin	Grade change	Baseline GGT ($N = 0$–60 U/l)	Peak GGT	Grade change	Baseline alkaline phosphatase ($N = 30$–110 U/l)	Peak alkaline phosphatase	Grade change
patient 8	16	458	0 to 4	202	425	2 to 3	192	295	2 to 2
patient 6	14	74	0 to 3	456	2048	3 to 4	415	1025	2 to 3

	Baseline ALT ($N = 0$–55 U/l)	Peak ALT	Grade change	Baseline AST ($N = 0$–45 U/l)	Peak AST	Grade change	No. of days on treatment
patient 8	204	264	2 to 2	91	361	1 to 2	56
patient 6	66	257	1 to 2	72	248	1 to 2	13

Grade change depicted represents CTC grade at baseline and the peak CTC grade; *N* represents normal laboratory range.
GGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTC, Common Terminology Criteria for Adverse Events.

Lyprinol® was relatively well tolerated with dyspeptic symptoms and anaemia being the most common complaint. The anaemia was normochromic normocytic and most probably disease related. However, the grade 3/4 liver dysfunction seen in two patients needs to be noted. One of them had significant hepatic involvement with disease, which most likely was the major reason for hepatic dysfunction. However, the second patient had no disease involvement of the liver and had normal liver function at baseline. This finding is of concern with the drug being possibly responsible for hepatic dysfunction. This has to be considered while evaluating patients who take Lyprinol® as a complementary medicine or while designing future studies with this agent.

None of the patients had evidence of objective tumour response. The significance of transient stabilisation of PSA in five patients is not clear. These patients were simultaneously on hormonal manipulation regimens that make it difficult to interpret the exact significance of this finding. Pain stability may have been a result of an anti-inflammatory effect. However, this is difficult to establish conclusively, especially since we used an unvalidated pain score.

There was no evidence from the available data that administration of the drug caused lipid profile abnormalities in the short term. The effect of long-term Lyprinol® as used in inflammatory conditions cannot be elucidated by this trial, due to the short treatment time.

In conclusion, it would be reasonable to state that Lyprinol® is a well-tolerated medication in the setting of advanced prostate and breast cancers. However, it does not appear to have any significant anticancer activity in this population. Patients who are using Lyprinol® as complimentary medication should be monitored for hepatic dysfunction. This study further highlights the importance of scientifically testing potential therapeutic agents, before widespread usage, based on media speculation.

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