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RESEARCH ARTICLE

Green-lipped mussel (*Perna canaliculus*) extract efficacy in knee osteoarthritis and improvement in gastrointestinal dysfunction: a pilot study

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Abstract
Objective Clinical data demonstrating efficacy for nutraceutical compounds marketed for the symptom relief of osteoarthritis (OA) have been largely contentious. Furthermore, no association has been linked between clinical trial inconsistencies and gastrointestinal (GI) dysfunction. The aim of this study was to primarily investigate the efficacy of a high-dose New Zealand green-lipped mussel (GLM) extract in patients diagnosed with OA of the knee and concurrently assess GLM impact on GI function.
Methods An open label, single group allocation study was conducted, that administered 3,000 mg/day of GLM extract over 8 weeks to 21 subjects diagnosed with knee OA. Outcome measures were scored using the WOMAC, the Lequesne algofunctional index, and the Gastrointestinal Symptom Rating Scale (GSRS) tools. An intention-to-treat analysis was employed and subject data collected at T_0, T_4 and T_8 weeks.
Results Paired t tests showed significant improvement for the Lequesne, WOMAC ($p < 0.001$) and GSRS ($p = 0.005$) scores. A repeated measures ANOVA analysis showed significant improvement in scores for the Lequesne ($F = 20.317$, $p < 0.001$), WOMAC ($F = 28.383$, $p < 0.001$) and the GSRS ($F = 9.221$, $p = 0.002$).
Conclusion Green-lipped mussel significantly improved knee joint pain, stiffness and mobility. We report for the first time that the administration of GLM extract also significantly improved GI symptoms by 49% in OA patients. Given that GI dysfunction is linked to analgesic medication use, we further conclude that the therapeutic efficacy of the GLM extract used was possibly correlated to its effects on GI function by improving GSRS scores from baseline. Results from this trial highlight the requisite for further clinical investigations of gastrointestinal tract function in OA patients.

Key words Green-lipped muscle extract · Osteoarthritis · Gastrointestinal function

Introduction

Osteoarthritis (OA) is the most prevalent form of arthritis and commonly targets the joints of the knee and less frequently joints of the hip, shoulder, spine (most commonly zygapophyseal, apophyseal, or facet joints of the spine, especially in the mid and lower cervical spine, and in the lower lumbar spine, L3 to L5) hands and toes (Buchanan and Kean 2002a, b; Wieland et al. 2005). With no known cure or proven disease-modifying therapy, the first-choice recommended pharmacological treatment for mild to moderate pain of the knee or hip is acetaminophen, with a maximum dose of up to 4 g/day (Buchanan and Kean 2002c). In the absence of a beneficial response, or pain and inflammatory sequelae, alternate therapy with an NSAID is indicated. Gastrointestinal (GI) safety however, may be compromised with long-term intake of these medications as they are associated with gastric or peptic ulcers (Garcia-Rodriguez et al. 1998; Roderiguez and Hernandez-Diaz

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2001), irritable bowel syndrome (IBS) (Locke et al. 2000) including dyspepsia (indigestion as discomfort experienced in the upper abdomen) (Rahme et al. 2002), chronic constipation (Chang et al. 2007), diarrhoea (Etienney et al. 2003) and morphological changes such as intestinal ulceration and increased GI inflammation and permeability (Scarpignato and Hunt 2010; Roderiguez and Hernandez-Diaz 2001).

Approximately 40% of the OA patients will self-medicate with dietary supplements, often using them exclusively (22%) or in combination (16%) with pharmaceutical medications i.e. analgesics (Armstrong et al. 2011). Clinical efficacy of nutraceuticals for the treatment of OA however remains collectively inconsistent, impacting the recommendation of their routine use. Popular supplements marketed towards OA include glucosamine, chondroitin and green-lipped mussel (GLM) extract (*Perna canaliculus*), and although clinical trial results variably support their use in OA, methodological flaws and inconsistent results preclude the recommendation of such compounds as an alternative treatment option (Brien et al. 2008; Wandel et al. 2010). In addition to analgesic use, GI function and integrity may possibly be compounded further by the presence of inflammation (Peuhkuri et al. 2010) and by the underlying rheumatic disease (Chong and Wang 2008).

The aim of this study was to investigate the therapeutic effect of a New Zealand GLM extract (GlycOmega™ PLUS) in patients diagnosed with OA of the knee(s) in relation to knee pain, stiffness and function. Furthermore, we examined GI symptoms and analgesic medication intake, and the interaction these may have on the therapeutic outcome of the GLM. It is therefore plausible to hypothesise that clinical efficacy of GLM in the treatment of knee OA may be associated with improved GI integrity and function. Understanding factors that impact digestion, absorption, metabolism and the health response process is crucial for the proper interpretation of biological responses, or the lack thereof, in clinical research (Possemiers et al. 2011).

Patients and methods

Participants

The study group comprised 23 patients with knee OA (9 males, 14 females) who satisfied the inclusion and exclusion criteria. Patients were recruited if their knee OA was confirmed by a Rheumatologist and they were not taking any form of herbal/multivitamin/nutritional supplements 4 weeks prior to recruitment. Patients were excluded if they had uncontrolled systemic disease, were pregnant or breast feeding, or had allergies/intolerances to shellfish. Participants were recruited from the Rheumatology Clinic at the Princess Alexandra Hospital, Brisbane from July 2010 to March 2011.

All 23 patients recruited satisfied the decision tree format of the ACR classification criteria for idiopathic clinical OA of the knee (Altman et al. 1986). The ACR clinical criteria for knee OA were fulfilled by the uniform presence of knee pain and at least 3 of the following 6 criteria: $\geq$ 50 years of age, morning stiffness lasting $\leq$30 min, crepitus on movement, bony tenderness, bony enlargement and no palpable warmth of synovium.

Each patient received written and verbal explanations regarding their involvement in the study before signing informed consent. The study protocol was in compliance with the Helsinki Declaration and was approved by the Human Research Ethics Committees of The University of Queensland and The Princess Alexandra Hospital.

Procedures

Patient demographic data and medical history were obtained at baseline (T_0). Anthropometric measurements, blood pressure and outcome measures were performed at T_0, T_4 and T_8 weeks, and blood samples for safety measures were collected at T_0 and T_8.

Anthropometric measurements

Height was measured with the subject standing barefoot using a body meter measuring tape with wall stop. Body mass was measured using calibrated scales and body mass index (BMI) was calculated using the formula—mass divided by height squared (kg/m^2); waist hip ratios were calculated by dividing the waist circumference (cm) by the hip circumference (cm).

Outcome measures

The Western Ontario McMaster Universities Arthritis Index (WOMAC) (Bellamy 2002) and the Lequesne algofunctional index (Lequesne et al. 1987; Lequesne and Maheu 2003) were designated the primary outcome measures and were administered via interview format by the clinical researcher at each participant's visit (T_0, T_4 and T_8). The WOMAC is a validated questionnaire designed for the assessment of lower extremity pain and function in OA of the knee or hip by assessing severity of knee pain (5 questions), stiffness (2 questions) and limitation of physical function (17 questions) (Bellamy 2002). Maximum scores of severity for each subscale are 20, 8 and 68, respectively. The maximum total score of severity for the WOMAC is 96. The Lequesne algofunctional index includes the measurement of pain (five questions), walking distance (one question), and activities of daily living (four questions),

with versions available for the hip and knee (Lequesne et al. 1987; Lequesne and Maheu 2003). Scores for each question are added together to provide a combined disease severity score. Scores of 1–4 are classified as mild OA, 5–7 moderate, 8–10 severe, 11–13 very severe, and 14 as extremely severe OA. Secondary outcome measures utilised the Gastrointestinal Symptom Rating Score (GSRS) questionnaire (Svedlund et al. 1988) and the SF-12v2^{TM} health survey (Tucker et al. 2010) via interview format. GI function was assessed using the validated GSRS questionnaire which contains 15 items designed to evaluate abdominal pain, gastro-oesophageal reflux, indigestion, diarrhoea and constipation. Each question is rated 0 to 3 in regards to intensity, frequency, duration, request for relief and impact on social performance. General quality of life was assessed using the SF-12 that includes 12 questions with results expressed in terms of 2 meta-scores: the physical component summary (PCS) and the mental component summary (MCS) (Ware et al. 1996; Tucker et al. 2010).

Assessment of safety

Blood pathology was performed to assess safety (full blood count, electrolytes and liver function tests). The inflammatory markers, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also included. Adverse events were documented by patients in diaries provided and events were recorded.

Intervention

Patients were allocated to 3,000 mg/day (3 × 500 mg b.i.d) of a proprietary blend of freeze-dried greenshell mussel meat (*Perna canaliculus*), GlycOmega^{TM} PLUS (Aroma NZ Ltd), which was dispensed in opaque white bottles for the 8-week duration. Compliance was monitored from participant recordings of daily intake of GLM in diaries provided.

Daily pain and rescue medication recordings

Participants were asked to complete two diaries that recorded daily intake of GLM, daily knee pain experienced on a 5 point Likert scale (T_0–T_4 and T_4–T_8) and daily rescue medication intake for knee pain.

Statistical analysis

The data in this clinical trial were treated according to an intention-to-treat analysis. Participants were included in the analysis if they had returned at least one diary (e.g. visit 2). Paired samples *t* tests were conducted for significant or marginally significant interactions. These were performed to investigate the difference in pre- and post-supplementation scores. Repeated measures analysis of variance (ANOVA) was conducted to investigate the changes between the time periods. The significance level was set at $p < 0.05$. Normality tests were performed for all the scoring variables to confirm the assumption of normality, justifying the use of *t* tests and analysis of variance tests.

Results

Patient characteristics

Three patients withdrew from the trial with 1 patient providing their first diary, resulting in a total of 21 participants (8 males and 13 females) with an age range from 41 to 87 years (mean ± SD, 61.1 ± 12.2 years) that provided data for an evaluable analysis. Weight and BMI did not change significantly between time points (mean ± SD, $T_0 = 96.9 \pm 27.1$ kg; $T_8 = 96.7 \pm 27.5$ kg) and (mean ± SD, $T_0 = 34.0 \pm 9.0$ kg/m^2; T_8=34.5 ± 9.2 kg/m^2), respectively. Waist to hip ratio remained unchanged between T_0 and T_8 (mean ± SD, 0.9 ± 0.1). Current medications were allowed to continue being taken through the trial period and included anti-cholesterol agents ($n = 6$), anti-diabetic agents ($n = 2$), diuretic ($n = 1$), calcium channel blockers ($n = 3$), beta-blockers ($n = 1$), angiotensin II receptor agonists ($n = 3$), ACE inhibitor ($n = 3$), anti-inflammatory medications ($n = 8$), proton pump inhibitors ($n = 7$), anti-depressant medications ($n = 9$), thyroid medications ($n = 3$), anti-histamine medication ($n = 2$), anti-coagulant medications ($n = 1$), gout medication ($n = 1$) and herpes medication ($n = 1$). Recent intake of antibiotics was reported by 7 patients, while 12 patients reported influenza vaccinations.

Dropouts

There were 3 dropouts in total from the original 23 patients recruited, 2 males and 1 female. These participants were lost to follow-up due to adverse events (2 males) and lack of interest in the trial (1 female). One of the male dropouts did return his first diary and data were included for analysis as intention-to-treat.

Outcome measures

Paired *t* tests were performed on the Lequesne, WOMAC (total and subscores), GSRS, and SF-12 (PCS and MCS scores) between T_0 and T_4, T_4 and T_8, and T_0 and T_8. Lequesne and WOMAC (total) showed significant changes between all intervals ($p < 0.001$) with WOMAC subscores of pain ($p < 0.001$), stiffness ($p = 0.002$) and physical

function ($p < 0.001$) indices all statistically significant; GSRS showed significant changes from T_0 to T_4 ($p = 0.004$) and from T_0 to T_8 ($p = 0.005$). MCS scores significantly changed from T_0 to T_4 ($p = 0.012$) only and PCS scores were not significant. The percentage change in scores demonstrated significant efficacy on all outcome measures (Table 1). A repeated measures ANOVA that was performed over the three time points gave significant results for all scores except PCS ($F = 0.364$, $p = 0.649$). Lequesne and WOMAC score differences were particularly strong ($p < 0.001$) as was the GSRS ($p = 0.002$) (Table 1).

Rescue medication

Rescue medication for knee pain associated with OA was allowed and recorded daily by the participants. Of the 21 patients, 7 did not use any rescue medication over the 8-week trial period, while 14 did and this included panadol (paracetamol dose 500 mg), panadol osteo (paracetamol 665 mg), panadeine forte (paracetamol 500 mg + codeine phosphate 30 mg), prednisone and various NSAIDs (aspirin 100 mg; indomethacin 25 mg; diclofenac sodium 50 mg; ibuprofen 200 mg].

Safety and adverse events

All blood parameters remained normal throughout the trial. CRP and ESR values did not significantly change over the 8-week period (mean ± SD $T_0 = 4.08 \pm 3.49$ mg/L; $T_8 = 3.31 \pm 2.69$ mg/L) and (mean ± SD $T_0 = 14.59 \pm 8.61$ mm/h; $T_8 = 14.21 \pm 7.66$ mm/h), respectively. Blood pressure remained stable throughout duration of the trial (mean ± SD T_0 = systolic 130.4 ± 18.3 mm Hg and diastolic 80.8 ± 11.8 mm Hg; T_8 = systolic 125.8 ± 17.3 mmHg and diastolic 76.0 ± 11.3 mm Hg). Adverse events included reflux ($n = 1$); abdominal pain, reflux and diarrhoea ($n = 1$) and gout ($n = 2$).

Discussion

We confirm that a standardized high dose (3,000 mg/day) GLM preparation used in this study was efficacious in treating OA symptoms of the knee. The improvement in symptom management from baseline was robustly significant for knee pain, stiffness and mobility.

A credible biological anti-inflammatory mechanism of action is reported for GLM in arthritic animal models, which have also demonstrated gastro-protective effects against NSAIDs-induced GI damage (Rainsford and Whitehouse 1980). A GLM-induced gastro-protective mechanism was noted in our sample group. Despite plausible mechanisms for the treatment of OA as an inhibitor of the lipoxygenase and cyclo-oxygenase pathways, a recent systematic review calls for further methodical investigations to provide clear evidence of efficacy, particularly

Table 1 Change in primary and secondary outcome measures from baseline (T_0) to week 4 (T_4) and from baseline to week 8 (T_8)

Outcome	Time	Mean	±SD	ES-Cohen's *d* (correlation)	*t* value	95% CI	*p* value
Lequesne Index	T_0	13.16	5.16				
	T_4	10.30	6.04	1.021 (0.875)	3.94	1.21, 3.94	0.001
	T_8	9.13	5.79	1.413 (0.864)	5.72	2.38, 5.12	<0.001
WOMAC (total)	T_0	41.63	19.75				
	T_4	30.0	17.30	1.074 (0.829)	4.10	5.08, 15.72	0.001
	T_8	22.80	17.57	1.957 (0.867)	7.78	12.87, 22.33	<0.001
WOMAC (pain)	T_0	8.64	3.99				
	T_4	4.90	3.34	1.745 (0.829)	5.34	2.13, 4.87	<0.001
	T_8	3.20	3.21	1.805 (0.649)	7.33	3.72, 6.68	<0.001
WOMAC (stiffness)	T_0	3.82	1.74				
	T_4	2.85	1.69	0.669 (0.643)	3.01	0.30, 1.70	0.007
	T_8	2.35	1.63	0.816 (0.428)	3.63	0.63, 2.37	0.002
WOMAC (physical)	T_0	29.18	14.96				
	T_4	22.25	12.91	0.879 (0.840)	3.20	2.04, 9.76	0.005
	T_8	17.25	13.70	1.877 (0.901)	7.38	7.81, 13.99	<0.001
GSRS	T_0	7.41	6.57				
	T_4	3.15	3.92	0.795 (0.478)	3.28	1.39, 6.31	0.004
	T_8	3.45	3.14	0.843 (0.532)	3.21	1.23, 5.87	0.005

GSRS gastrointestinal symptom rating score, *ES* effect size (Cohen's *d*), Paired *t* tests between T_0 and T_4 scores and T_0 and T_8 scores and 95% CI for the differences

optimal dosing (Brien et al. 2008; Whitehouse et al. 1997). In that review, doses in the range of 1,050–1,150 mg/day were reported. In this study, we report that a high dose of 3,000 mg/day was efficacious and attenuated pain, stiffness and joint mobility. Efficacy was already established at 4 weeks of supplementation with significant reductions in pain scores and was maintained when assessed at week 8 (Table 1). The high dose of the GLM employed in this pilot trial may provide additional information for future controlled phase IIb dosing studies.

We acknowledge that a weakness of this study is the lack of a placebo comparator (Zhang et al. 2008). However, as a preliminary study, it provides additional clinical evidence to support the efficacy of GLM and to formulate a hypothesis that has not yet been previously investigated, namely GI dysfunction in OA. GI dysfunction in clinical trials investigating nutraceutical efficacy can be a significant limiting factor. Prescribed analgesic medications can disrupt GI function (Wolfe et al. 1999; Chang et al. 2007; Etienney et al. 2003; Scarpignato and Hunt 2010; Roderiguez and Hernandez-Diaz 2001) and hence this may significantly affect nutraceutical metabolism, absorption and efficacy.

We have demonstrated that GLM was efficacious in improving OA symptoms and function, and that it may have important GI sequelae that assist with GLM utilization and hence efficacy. GI dysfunction was significantly improved with study progress in all subjects.

Ethical approval and clinical trial registration

Approval for this prospective study was obtained from the Ethics Committee of The University of Queensland and Princess Alexandra Hospital Human Research Ethics Committees. The clinical trial was registered with the Australian and New Zealand Clinical Trail Registry (ANZCTRN: 12611000517976).

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Conflicts of interest Funding and study medication for the project was received from the clinical trial sponsor Aroma New Zealand Ltd. The sponsor had no involvement in the collection, analysis or interpretation of the data; writing the report; or the decision to submit the paper for publication.

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