An aqueous formulation of γ-linolenic acid with anti-proliferative action on human pancreatic cancer cell lines

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Essential fatty acids, especially γ-linolenic acid (GLA), have been shown to directly inhibit the growth of cancer cell lines in culture. The aim of this study was to see whether an aqueous formulation of GLA works as well as the lithium-based salt. We evaluated the effect of the 1-deoxy-1-methylamino-D-glucitol salt of GLA (MeGLA) on the growth of two human pancreatic cancer cell lines (Panc-1 and MIA PaCa-2) *in vitro*, and compared its effects with a previously studied formulation, lithium GLA (LiGLA). The effect of time exposure (2–7 days) and difference in concentration (0–1000 μmol/l) were studied using 96-well culture plates. Cell growth was assessed by MTT assay. Control experiments were performed with meglumine alone in similar concentrations. MeGLA had cytostatic and cytotoxic effects on pancreatic cancer cell lines with 50% growth inhibition at 30–100 μmol/l and cytotoxic effects at 60–250 μmol/l. The degree of growth inhibition increased with time of exposure to MeGLA. The anti-proliferative effects of MeGLA were similar to those previously observed with LiGLA. We conclude that MeGLA has equivalent anti-proliferative activity to LiGLA when tested *in vitro* against pancreatic cancer cell lines and is therefore a suitable alternative to LiGLA for investigation of the *in vivo* activity of GLA against pancreatic adenocarcinomas. *Anti-Cancer Drugs* 15:157–160 © 2004 Lippincott Williams & Wilkins.

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Introduction

Advanced pancreatic cancer is a disease with a mortality rate that is the same as its incidence [1]. Standard chemotherapy has not improved survival figures over the last 30 years. Conventional regimes are hampered by toxicity with relatively small gains in survival or quality of life. A regime which combines low toxicity and improved outcome either in terms of survival or enhanced quality of life is desperately needed.

Essential fatty acids (EFA) have been studied extensively and have cytotoxic and growth inhibitory effects *in vitro* against a wide variety of cell lines. EFAs also appear to have anti-metastatic effects, anti-cachetic effects and can decrease the resistance of malignant cells to conventional chemotherapeutic agents [2]. These effects are seen *in vitro* at very low concentrations that have no effect on normal cells.

LiGLA is a lithium-based salt of γ-linolenic acid (GLA) that is poorly soluble in water and clinically employed in emulsion or liposomal formulations. These oily preparations have dose-limiting side effects that can be overcome by an aqueous formulation of GLA. MeGLA is based on meglumine as the carrier molecule for GLA. This is a water-soluble preparation and meglumine is extensively used in common water-soluble contrast media. It is hoped that this preparation will be better tolerated *in vivo*.

Previous work has indicated that *in vitro* the LiGLA preparation is cytotoxic to pancreatic cell lines [3]. Direct injection of LiGLA in high doses to an experimental tumor stops tumor progression and in a proportion of cases the tumor regressed [4]. In this study, two preparations of the essential fatty acid GLA were tested *in vitro* against two established human pancreatic cell lines, MIA PaCa2 and Panc-1.

Materials and methods

LiGLA (Scotia Pharmaceuticals, Stirling, UK) was supplied in amber vials containing 5 ml of aqueous solution with 140 mg LiGLA/ml in 12.75% ethanol. MeGLA (Scotia Pharmaceuticals) was supplied in 1-ml clear ampoules containing 5 mg/ml MeGLA in water. All stock was kept in a refrigerator at 4°C.

The two pancreatic ductal carcinoma cell lines Panc-1 and MIA PaCa-2 (European Collection of Animal Cell Cultures, Salisbury, UK) were established in Dulbecco's modified Eagle's medium with added 3–10% fetal bovine serum (FBS) and L-glutamine antibiotic solution 1% vv (referred to as standard medium) (all from Sigma, Poole,

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UK). Cell cultures were incubated at 37°C in 5% CO_2 in humidified air (standard conditions).

Cells were harvested using trypsin–EDTA, counted with a hemocytometer and seeded at a density of 2500 cells/well in 100 µl of medium in 96-well flat-bottomed cell culture plates. The cells were incubated for 24 h in order that they establish themselves and adhere to the culture plates.

MeGLA or LiGLA was added to the culture wells in 50 µl of standard medium at drug concentrations of between 3.4 to 1000 µmol/l. Medium-only controls were included with each experiment. Control wells contained meglumine in equivalent concentrations to those used for MeGLA. Plates were inspected daily for signs of confluence and discarded if this occurred. At 7 days after addition of the fatty acids the plates were removed from the incubator and the percentage of live cells was estimated using an MTT assay [3].

Time course studies were performed with LiGLA (3.9–1000 µmol/l), MeGLA (3.4–870 µmol/l) and meglumine (3.4–870 µmol/l). Plates were seeded and sequentially removed and read over a period of 7 days.

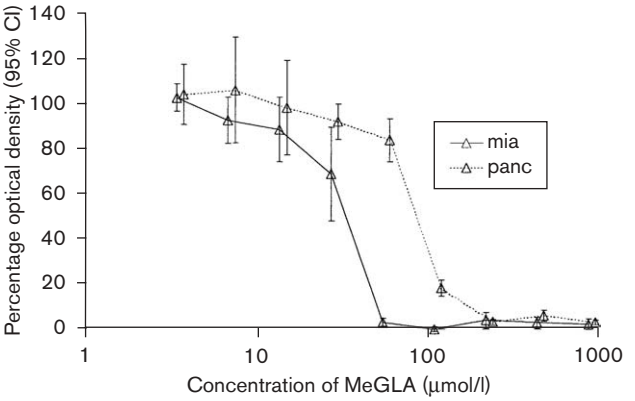
Growth inhibition was calculated as the percentage optical density of test wells compared to control wells (cells with culture medium only). Statistical analysis was performed by the Mann–Whitney *U*-test.

Results

Cytotoxicity studies

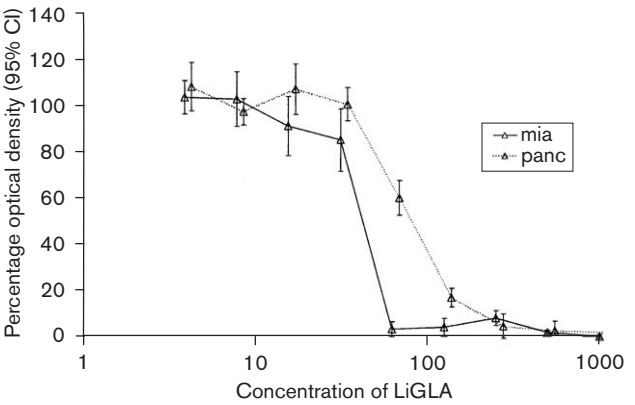
Both MeGLA and LiGLA in high concentrations (above 500 µmol/l) caused cellular disruption within 24 h of addition to both pancreatic cancer cell lines. Concentrations of between 15 and 250 µmol/l were growth inhibitory to the cell lines. At these concentrations cytotoxicity was not observed. Very low concentrations (less than 10 µmol/l) appeared to confer a small growth advantage to the cells (Figs 1 and 2). Control experiments with meglumine did not show any effect at similar concentrations.

Fig. 1



The effect of MeGLA on the growth of Panc-1 and MIA PaCa-2 cell lines. The growth of cells exposed to MeGLA for 7 days is expressed as a percentage of the growth of cells in medium-only control wells. Mean of six to 12 separate observations, each consisting of nine test wells. Vertical bars represent 95% confidence intervals (CI).

Fig. 2



The effect of LiGLA on the growth of Panc-1 and MIA PaCa-2 cell lines. The growth of cells exposed to LiGLA for 7 days is expressed as a percentage of the growth of cells in medium-only control wells. Mean of six to 12 separate observations, each consisting of six to nine test wells. Vertical bars represent 95% confidence intervals (CI).

Time course studies

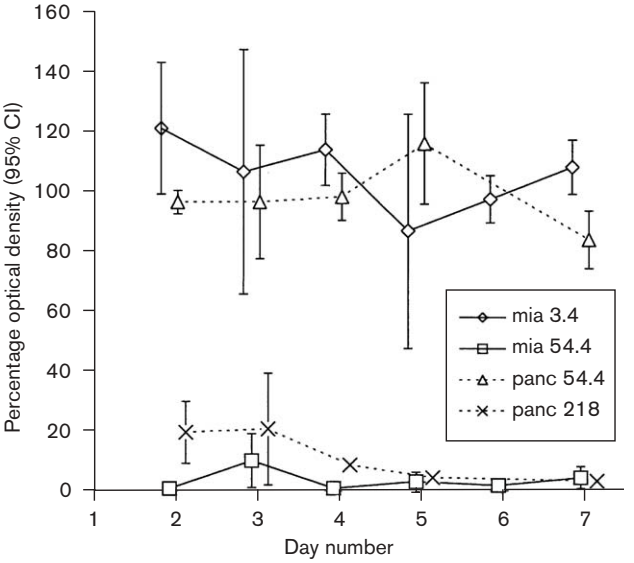
Within the growth inhibitory concentration range a pronounced time-dependent effect was not seen with either substance (Figs 3 and 4). An apparent reduction in growth inhibition by LiGLA at high concentration on day 4 was not significantly different from day 2 and 3. Meglumine on its own had no effect on the growth of cell lines.

The effects of MeGLA were similar to those of LiGLA and were concentration-dependent. The 50% growth inhibition concentration (IC_{50}) for the Panc-1 cell line was 80 µmol/l for both LiGLA and MeGLA, and for the MIA PaCa-2 cell line was 40 µmol/l for both substances.

Discussion

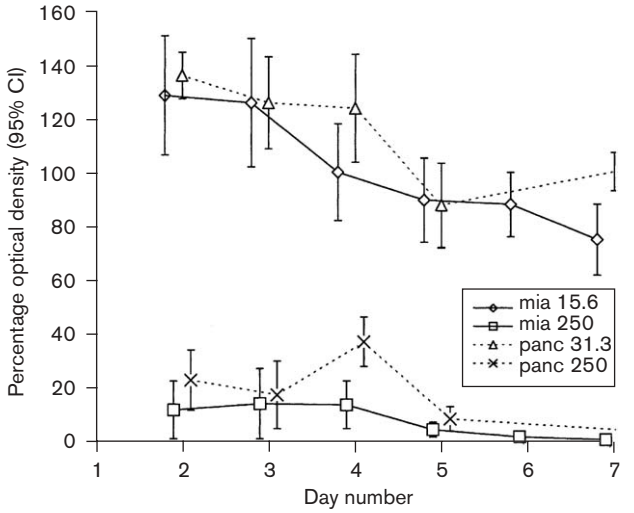
This study has shown that two different formulations of GLA (LiGLA and MeGLA) have equivalent growth inhibitory effects on pancreatic cancer cell lines in culture. These observations are important because MeGLA is a new formulation designed to simplify

Fig. 3



Time course study of the effect of MeGLA on MIA PaCa-2 cells (3.4 and 54.4 μmol/l) and Panc-1 cells (54.4 and 218 μmol/l). The growth of cells exposed to MeGLA is expressed as a percentage of the growth of cells in medium-only control wells. Mean of six to 12 separate observations. Vertical bars represent 95% confidence intervals (CI).

Fig. 4



Time course study of the effect of LiGLA on MIA PaCa-2 cells (15.6 and 250 μmol/l) and Panc-1 cells (31.3 and 259 μmol/l). The growth of cells exposed to LiGLA is expressed as a percentage of the growth of cells in medium-only control wells. Mean of six to 12 separate observations. Vertical bars represent 95% confidence intervals (CI).

delivery of γ-linolenate *in vivo*, and these compounds appear to offer a potential for anti-tumor therapy, provided adequate local concentrations can be achieved.

LiGLA is a poorly soluble fatty acid salt which has been formulated in a liposomal form in an effort to deliver higher doses systemically. High doses of LiGLA are an irritant to endothelial tissues and there have been problems with catheter blockage when given as infusions. By comparison, MeGLA is a readily water-soluble formulation based on meglumine, a common component of non-ionic i.v. contrast media. It is anticipated that MeGLA will not show the irritant effects of LiGLA on the vascular endothelium and peritoneum that, up to now, have limited the maximum dose of GLA that can be delivered. The meglumine salt of GLA overcomes the potential problem of lithium toxicity associated with LiGLA.

Many papers have shown an *in vitro* growth inhibitory effect of GLA preparations in a wide variety of tumor cell lines [2]. It is widely agreed that increased lipid peroxidation is the most likely mechanism for this effect. Much higher concentrations of GLA have a detergent-like effect on malignant and normal cells, causing cellular disruption and death. EFAs bind to albumin and this reduces their capacity to influence cell growth [5,6]. This may explain why a phase III study showed survival was not different between the groups receiving low-dose i.v. LiGLA and oral LiGLA [7]. In contrast, if GLA can be applied without exposure to plasma, encouraging responses are seen. Intra-vesical application to transitional cell carcinoma is promising [8,9]. Likewise, direct application to tumors appears effective [4,10,11] and in experimental systems, direct injection of GLA into implanted human tumors in nude mice can lead to tumor regression [4].

GLA has some attractions for the treatment of pancreatic cancer. It is not as toxic as standard chemotherapy, but hemolysis is a potential problem when rapid i.v. infusion is used [12]. Hemolysis could be avoided by local direct injection and this would also have the advantage of achieving high concentrations of the agent to the tumor, overcoming the problems of drug dilution and binding to albumin associated with systemic therapy. Pancreatic cancer is amenable to such direct approaches either by direct tumor injection (using computed tomography guidance) or by intra-arterial injection, as already described for chemotherapy agents [13].

MeGLA has significant growth inhibitory effects at micromolar concentrations. The concentrations needed to produce these effects are similar to that recorded by other investigators [8,9,14]. Similar results were obtained with LiGLA [3]. Similar IC_{50}s have been seen by others working with glioblastoma cell lines [15]. These findings suggest that similar molar concentrations of GLA in different formulations can achieve similar effects. Findings with LiGLA can therefore be extrapolated with confidence to the MeGLA formulation, which in practice

is easier to handle and administer. However, the effects of prolonged exposure to MeGLA at lower concentrations are smaller than those seen with LIGLA, suggesting that prolonged contact with tumor cells may be less important than delivering an effective dose of MeGLA.

There have been no other studies published on the effects of MeGLA on pancreatic cell lines. The rationale for this study was to verify its effect on pancreatic cells as a prelude to *in vivo* studies. The results are encouraging and would suggest that further work is justified. However, previous *in vivo* experiments suggest a topical or loco-regional approach is likely to produce the best clinical effect.

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