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Anti-cancerous effect of linoleic acid and conjugated linoleic acid on hepatic cancer cells and histocytic lymphoma cells: *In vitro*

[1]Aruna Bhatia, [1]Arpit Sharma, [2]Praveen P. Balgir and [2]Deepak Kapoor

[1]Immunology and Immunotechnology Laboratory, Department of Biotechnology Punjabi University, Patiala, Punjab, India
[2]Genetic Engineering Laboratory, Department of Biotechnology Punjabi University, Patiala, Punjab, India

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ABSTRACT

Conjugated linoleic acid, derivative of linoleic acid has been reported in many in vitro and in vivo studies to have beneficial health effects. However, little information is available on dosage effect of this compound against cancerous cell lines. In this study, we investigated the anti-carcinogenic potential of CLA and LA using tretrazolium dye reduction test against HepG2 and U937 cancerous cell lines. Present study supports the role of both CLA and LA as anti-cancerous agents in preventing cancerous cell proliferation.

Keywords: Conjugated Linoleic acid, cancer, cell lines

INTRODUCTION

Studies related to CLA (Conjugated Linoleic acid) and LA (Linoleic acid) in previous years have demonstrated its potential against many cancerous cell lines i.e. Gastric adenocarcinoma[1], Human colon cancer[2], Human glioblastoma[3] and Human Breast cancer[4]. CLA is a mixture of positional and geometric isomers of linoleic acid, which are synthesized *in vivo* by rumen bacteria. At least 28 different CLA isomers have been identified. Of these, the 9cis, 11trans form is likely to be the most common natural form of CLA and it demonstrates biological effects[5]. The objective of the present study is to evaluate the comparative analysis of dose dependent effect of both these compounds against HepG2 (Hepatocellular carcinoma, human) and U937 (Lymphocyte, Myeloid) cancerous cell lines.

MATERIALS AND METHODS

Linoleic acid (18:2) was procured from Hi-Media Laboratories Pvt. Ltd. Mumbai, India and purified Conjugated linoleic acid (c9:t11) isomer was obtained after bioconversion of LA to CLA using *Bifidobacterium* 235 (procured from National Collection of Dairy Cultures (NCDC), Karnal, Haryana, India). Stock solution (1mg/ml) of LA and CLA was prepared using 1% Tween80. Further dilutions (10-100µg) were made using deionized water and stored at -4^0C until further use.

i. Cell Lines

HepG2 (Hepatocellular carcinoma, human) and U937 (Lymphocyte, Myeloid) were procured from National Centre for Cell Science (NCCS), Pune, India. For maintenance of cell line, cells were trypsinized and 5ml of complete media with 10% Fetal bovine Serum (FBS) and 2% antibiotic solution (100 U/ml penicillin, 100 mg/ml

streptomycin) was added to trypnised cells. Cell lines were routinely maintained and subcultured in 25 cm^2 plastic flasks at 37^0C in a humidified CO_2 incubator (95% air and 5% CO_2).

ii. Cell Proliferation Assay

The effect of LA and CLA preparations on cell proliferation was evaluated using methylthia- zolyldiphenyl-tetrazolium bromide (MTT) cell proliferation assays. This assay is based on the conversion of the yellow tretrazolium dye MTT to purple formazan crystals by metabolically active cells. Briefly, HepG2 and U937 cells were seeded in 96-well tissue culture plates and maintained overnight in RPMI 1640 and DMEM plus 10% FBS. Cells were treated with various concentrations of LA and CLA. Cell number was calculated using haemocytometer and diluted to 75,000 cells/ml. Final volume was kept 100µl after treating cells with different conc. of test compound. 20µl of 5mg/ml MTT was added to each well and incubated for 3.5 hr at 37˚C in culture hood. Media was removed and dye crystals were dissolved using DMSO (Dimethyl sulfoxide). Absorbance was read at 590nm with a reference filter of 620nm on microplate reader, (Biorad 680, USA).

Calculations:

$$\% \text{ Cell Viability} = \frac{\text{OD Sample} - \text{OD cell free sample blank}}{\text{OD Medium Control}} \times 100$$

iii. Statistics:

Values were expressed as mean ± S.D. The statistical significance of the differences was assumed at $p<0.05$.

RESULTS AND DISCUSSION

Anti-cancerous activity of both CLA (c9:t11) and LA was analyzed using MTT dye reduction assay at concentration from 10-100 µg/ml. It was observed that CLA produces prominent reduction in cell viability of HepG2 (48.98%↓) as compared to U937(26.65%↓). However LA showed marginally higher reduction in U937 cells (41.51%↓) than in HepG2 (38.94%↓) cells (Figure 1-3). Both the compounds reduced the cell viability in a concentration dependent manner. Maximum reduction was observed with conc. 50-100 µg/ml in HepG2 cells and 70-100µg/ml in U937 cells with both the compounds. Anti-proliferative effect of CLA was found to be greater in HepG2 cells, whereas LA was found to be effective against U937 cells. To best of our knowledge, till date the anti-proliferative effect of LA has not been reported against myeloid cell line. In the previous studies, major effect of LA has not been observed against human colon adenocarcinoma Caco-2[7]. However cytotoxic effect has been observed against MDA-MB-231 and MCF-7 Human Breast Cancer Cells[8].

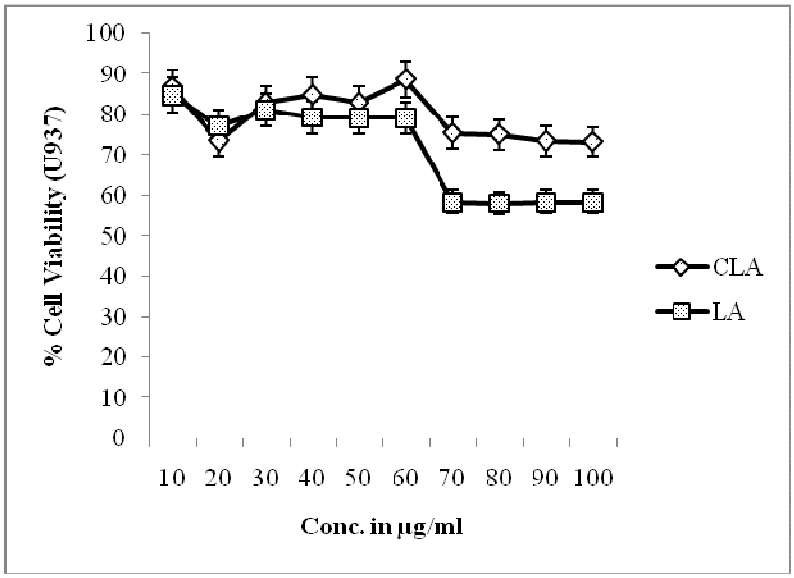

Figure 1. Effect of CLA (c9, t11) and LA of on U937 cancerous cell line after incubation at 37℃ for 3.5hr in 5%CO_2 chamber
Results are represented as mean ± S.D.

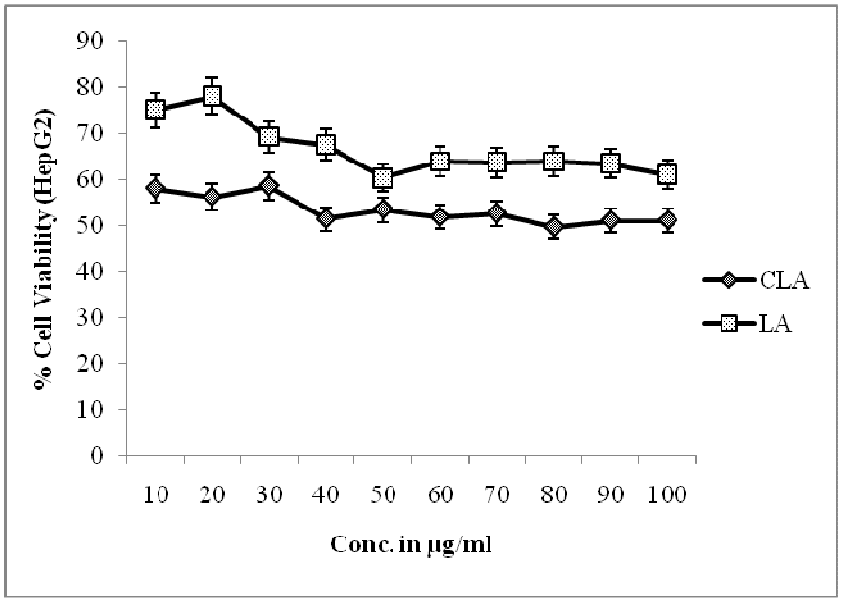

Figure 2. Effect of CLA (c9: t11) and LA of on HepG2 cancerous cell line after incubation at 37℃ for 3.5hr in 5%CO_2 chamber
Results are represented as mean ± S.D.

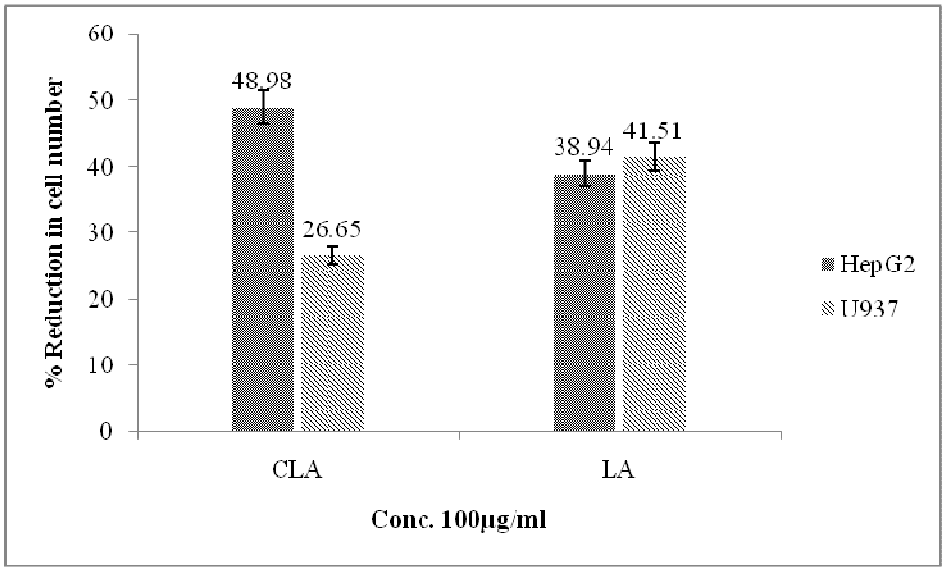

Figure 3. Comparison of percentage reduction in cell number in HepG2 and U937 at 100µg/ml of CLA (c9: t11) and LA after incubation at 37℃ for 3.5hr in 5%CO_2 chamber
Results are represented as mean ± S.D.

The anti-cancerous effect of LA can be contributed to its property to influence biological micro-environment to carry substances across cell membrane as studied in linoleic acid polyphase liposome[9]. CLA on the other hand has been found to have anti-cancerous effect against many cell lines[3,4,5,6], role of c9,t11-CLA has been observed *via* blocking the cell cycle, with reduced expressions of cyclin A, B1 and D1 and enhanced expressions of CDKI[1].

CONCLUSION

It is concluded that both Conjugated linoleic acid and Linoleic acid have anti-cancerous potential against HepG2 and U937 cancerous cell lines. Both the compound inhibited the growth of cancerous cells in a concentration dependent manner. However CLA showed better anti-cancerous potential against HepG2.

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