International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 25s,2025
https://theaspd.com/index.php

The Impact Of Acetyl-L-Carnitine In Modulating Viability And Inflammatory Cytokines In ACHN Renal Adenocarcinoma Cells

Ahmed Ibrahim Mohammed[2], Sanaz Sheikhzadeh[2], Mazdak Razi[3]
[1]Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[2]Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

*Corresponding author: s.sheikhzadeh@urmia.ac.ir, https://orcid.org/0000-0003-4809-5769

Abstract
Acetyl-L-Carnitine (ALCAR) is recognized for its roles in energy metabolism, neuroprotection, and modulation of inflammatory responses. The effects of ALCAR on cancer biology remain an emerging area of interest. This study investigates the impact of ALCAR on the ACHN renal adenocarcinoma cell line, focusing on cell viability and inflammatory cytokine regulation. ACHN cells were treated with ALCAR (0–2 μM) for 24 and 72 hours. ACHN cell viability was assessed via MTT test to determine the IC50. The levels of the pro-inflammatory cytokines IL-6, IL1β, and TNF-α were quantified using ELISA.
The results demonstrated that ALCAR significantly decreased ACHN cell viability in a time- and dose-dependent manner, resulting in IC_{50} values of 51.38 and 45.32 μM/mL at 24 and 72 hours, respectively. ALCAR also up regulated IL-1β and TNF-α levels significantly, while IL-6 levels showed no significant change.
In conclusion, the observed reduction in cancer cell survival highlights ALCAR's potential as a therapeutic agent, particularly in renal cell carcinoma. However, further research is required to elucidate the underlying mechanisms and optimize its application in targeted cancer therapies.
Keywords: *Acetyl-L-carnitine, ACHN cells, pro-inflammatory cytokines, cancer.*

INTRODUCTION

Kidney cancer represents 5% of all malignancies and ranks as the sixth most commonly diagnosed cancer in men. Renal cell carcinoma (RCC) originates from renal tubular epithelial cells and makes up more than 90% of kidney cancers (1). Approximately 2.4% of all adult malignancies are attributed to RCC. In 2020, this cancer was responsible for nearly 180,000 deaths worldwide (2). The incidence of RCC, which continues to rise, varies globally and is more prevalent in developed countries than in developing ones. Additionally, RCC occurs more frequently in men than in women. The death rate is also greater in men compared to women. The common symptoms of RCC include flank pain, hematuria, and a palpable abdominal mass. The most frequent sites of distant metastasis are the bones, lungs, and brain, while the opposite kidney, the adrenal glands, and the liver may also be affected (1).

RCC is traditionally regarded as an immunogenic tumor and is generally resistant to cytotoxic chemotherapy and radiation therapy (2). Surgery is still a critical component of treatment in RCC. While nephrectomy has traditionally been the standard procedure for the management of kidney tumors, the detection of small renal lesions and increasing evidence that surgery-related kidney disease can raise patient morbidity have led to a shift toward more conservative treatment strategies. Twenty to thirty percent of patients with RCC develop distant metastases, and two to five percent experience local recurrence, despite surgery being a viable treatment option. Therefore, adjuvant treatment following surgery is crucial (1).

L-carnitine is found in tissues and cells in the form of free carnitine and acylcarnitines, including acetylL-carnitine (ALCAR). The most recognized function of L-carnitine is its role as a central transporter of long-chain fatty acids toward mitochondria structure for β-oxidation. ALCAR is a small, water-soluble molecule that can be used to transport acetyl groups. ALCAR may enhance mitochondrial function and energetics, stabilize membranes, exhibit antioxidant activity, modulate protein and gene expression, and enhance cholinergic neurotransmission (3). This compound has also been used in cancer research studies and has demonstrated its effectiveness (4, 5).

Cytokines are functional proteins which are produced by the immune system (6,7). Pro-inflammatory cytokines particularly the Interleukins (IL)-1 family, tumor necrosis factor alpha (TNF-α), and IL-6 are play vital roles in establishing chronic inflammation within the tumor environment. These cytokines can exhibit anti-tumorigenic or pro-tumorigenic functions in accordance with factors such as the type of cancer, tumor microenvironment, and other related factors (8,9).

This study aims to explore how ALCAR influences the human renal adenocarcinoma (ACHN) cell line, with a particular focus on pro-inflammatory cytokines. The ACHN cell line is among the cell lines used in RCC research (10). The findings could provide valuable insights into the role of ALCAR in regulating inflammatory responses, potentially contributing to the find of novel therapeutic strategies for RCC.

MATERIAL AND METHODS:

Cells Culture

ACHN cell line was obtained from the Pasteur Institute (Iran). ACHN cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco-Invitrogen, Carlsbad, CA, USA) supplemented with 10% Fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin. The cells were kept at 37°C in a 95% humidified atmosphere with 5% CO_2. Cells were passaged if needed, and from the third passage, they were used for the experiment.

Cell Viability Assay

ALCAR was obtained from American International Lab, Inc. (GranadaHills, CA, USA). Cells were seeded individually in a sterile 96-well plate (1×10^5 cells per well) and incubated overnight in an incubator under standard conditions. After 24 hours, the medium was changed with serum-free DMEM and incubated for an additional 24 hours. The cells were then treated with diverse dilutions of ALCAR (0, 0.5, 1, 1.5, 2 µM) and incubated for 24 and 72 hours. The media were aspirated after incubation, and the cells were treated with MTT (Sigma, St. Louis, MO, USA) at a concentration of 5 mg/mL dissolved in phosphate-buffered saline. Furthermore, 10 µL of MTT was added to the wells, followed by incubation for an additional 4 hours at 37°C. The medium was aspirated, and 100 µL of dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) was added to wells, and the absorbance was measured at 570 nm using a microplate reader (DANA, 32000, Iran), and the half maximal inhibitory concentration (IC50) was calculated. Each test was performed 4 times in triplicate.

Cytokine Evaluation

In this study, we evaluated the concentrations of pro-inflammatory cytokine IL-6 (Elabsciences, USA, Cat N: E-EL-H6156), IL-1β (Elabsciences, USA, Cat N: E-EL-H0149), and TNF-α (Elabsciences, USA, Cat N: E-EL-H0109), using a standardized Enzyme-Linked Immunosorbent Assay (ELISA) protocol. A pre-coated 96-well microplate was used to promote cytokine binding. To each well, 100 µL of each dilution of the standard, samples, and blank were added. After incubation, the liquid was discarded from each well without washing. Then, 100 µL of biotinylated detection antibody working solution was added. The plate was incubated at 37°C for one hour.

After incubation, the liquid was decanted from wells, and 350 µL of wash buffer was added to them. The wells were soaked for 1 minute before the solution was aspirated or decanted, and the plate was gently patted dry with clean paper. This washing stage was repeated 3 times. Afterward, 100 µL of HRP conjugate working solution was added to the washed wells. The plate was incubated for another 30 minutes. The liquid was then removed from each well, and the wash procedure was repeated 5 times. Following the washes, 90 µL of substrate reagent was introduced into each well. The plate was sealed and incubated for approximately 15 minutes at 37°C, ensuring the plate was protected from light. After the incubation period, 50 µL of stop solution was added to each well. Finally, the optical density (OD) of each well was assessed at 450 nm using a microplate reader.

Statistical Analysis

The Levene's test and the Kolmogorov-Smirnov test were employed to assess the homogeneity of variance and the normality of the data distribution in the statistical analysis. Quantitative biochemical and molecular data were analyzed using two-way ANOVA, with post-hoc analysis performed using Tukey's multiple comparison test. Correlations and regressions were evaluated using SPSS software (version 11.00, California, USA). A p-value of <0.05 was considered statistically significant for all analyses. Data were presented as Mean ± SD, and graphs were created using GraphPad Prism software.

Results Cell Viability and Dose Determination

ACHN cells were treated with ALCAR at concentrations of 0, 0.5, 1, 1.5, and 2 µM/mL. As the concentration of ALCAR elevated, the viability of the ACHN cells was decreased (Figure 1). The IC50 of ALCAR was also determined to choose the optimal treatment dose and duration in subsequent studies. The ACHN cell line showed Absolute IC50 values of 51.38 and 45.32 after 24 and 72 h treatment, respectively.

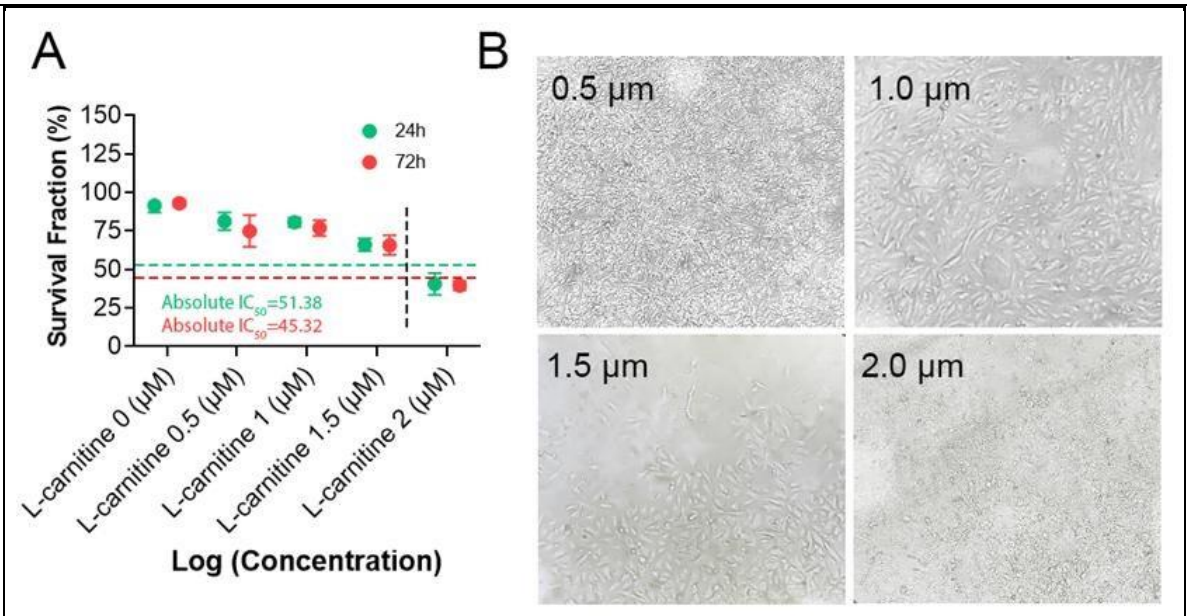

Figure 1: Effects of ALCAR on ACHN cells.

(A) Dose-response curve of ACHN cells following treatment with acetyl-L-carnitine (ALCAR) for 24 and 72 hours. (B) Photomicrographs of ACHN cells treated with 0.5, 1, 1.5, and 2 μM/mL of ALCAR. Cell viability was assessed at different time points for each treatment condition. The absolute IC50 is represented at the intersection of the red and green dashed lines with the black dashed. The concentration values were transformed to log scale, normalized to percentage, and averaged from four replicates.

Pro-inflammatory Cytokines

To investigate the effect of ALCAR on pro-inflammatory cytokine levels in ACHN cells, the levels of IL6, IL-1β, and TNF-α pro-inflammatory cytokines were assessed. The results demonstrated that ALCAR significantly ($p < 0.05$) increased IL-1β levels compared to untreated ACHN cells at both 24 and 72 hours post-treatment, with this elevation observed in a time-dependent manner (Figure 2A). Although ALCARtreated ACHN cells exhibited higher IL-6 levels compared to untreated ACHN cells, this elevation was not statistically significant ($p > 0.05$; Figure 2B). Regarding TNF-α, ALCAR treatment resulted in a significant ($p < 0.05$) increase in TNF-α levels compared to untreated cells at both 24 and 72 hours posttreatment (Figure 2C).

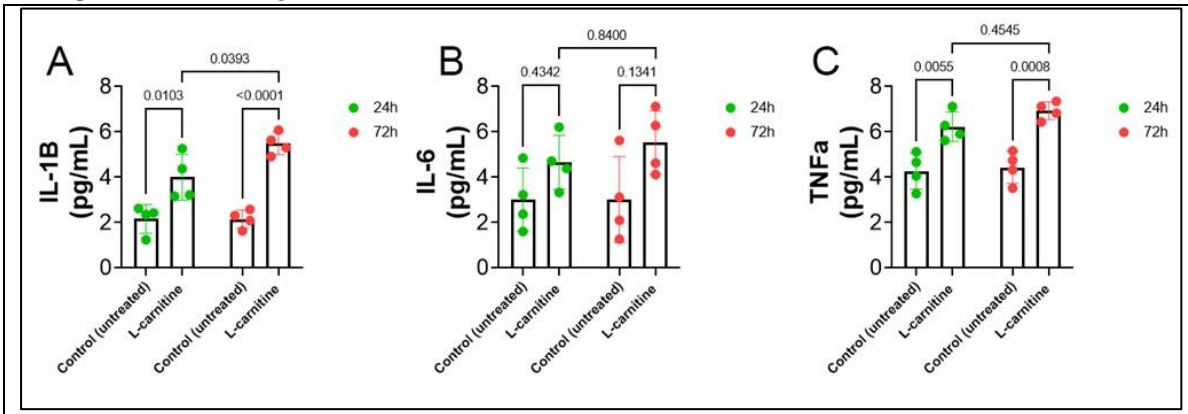

Figure 2: Effect of Acetyl-L-carnitine (ALCAR) on pro-inflammatory cytokines expression by ACHN cells; (A) mean changes in the IL-1β, (B) IL-6, and (C) TNF-α following 24 and 72 hours of ALCAR treatment. All data are presented in Mean±SD; $p<0.05$ was considered as a significant difference, n=4 sample/group.

DISCUSSION

ALCAR is involved in energy metabolism and exhibits neuroprotective and neurotrophic properties. It has minimal side effects and demonstrates excellent tolerability. While it is authorized as a dietary supplement in some countries, others have approved its use as a therapeutic drug (11).

Several lines of evidence suggest that ALCAR holds potential for use in cancer research. For instance, Baci et al. demonstrated the ability of ALCAR to suppress the angiogenesis and invasion of prostate cancer cells through the reduction in the levels of pro-inflammatory cytokines, chemokines, and MMP9 (5). Furthermore, Albogami et al. showed that the antitumor effects of ALCAR in HT29 and HepG2 adenocarcinoma cell lines are driven by the suppression of adhesion, migration, and invasion (4). Another study by Donisi et al. also demonstrated that ALCAR and L-Carnitine induce mitophagy-related cell death and metabolic alterations in colorectal cancer cells (HCT 116 and HT-29). They reported that these compounds significantly reduced cell viability, induced oxidative stress, and modulated bioenergetics (12). Moreover, another study highlighted that ALCAR exhibits significant anti-metastatic activity, both in

combination with a histone deacetylase inhibition or alone (11). These findings collectively underscore the promising role of ALCAR in cancer research.

Given the significance of renal cell carcinoma, we investigated the potential impact of ALCAR on the ACHN cell line. Here, we demonstrate that ALCAR reduced the viability of ACHN cells (Figure 1). This finding aligns with previous studies, which have suggested that ALCAR may have potential applications in cancer treatment (4, 5, 10, 11).

IL-6, IL-1β, and TNF-α pro-inflammatory cytokines were also measured and analyzed in ACHN cells following treatment with ALCAR in the present study. ALCAR up regulated IL-1β and TNF-α levels significantly, while IL-6 levels showed no significant change. Carnitine and its derivatives have been shown to be highly effective in modulating cytokines (12-15).

TNF-α is implicated in the regulation of various signaling pathways. In general, the TNF-α influences tumor developments through various mechanisms; Altered expression of TNF-α has been identified in some types of cancers, such as ovarian, prostate, breast cancer, and liver. Although TNF-α's antitumor effects have been harnessed for cancer therapy, it is noteworthy that this cytokine can also promote tumor progression (7). Contrary to our research, a decrease in TNF-α cytokine levels was noticed in prostate cancer cells following exposure to ALCAR (5). A meta-analysis and systematic review also demonstrated that L-carnitine supplementation decreased TNF-α levels in the serum of adults with inflammation, thereby reducing the inflammatory state in this population (15). ALCAR has also been demonstrated to reduce the expression of TNF-α in rats with atherosclerosis (16). IL-1β exhibits diverse effects in cancer (17). Findings suggest a pro-tumorigenic role for IL-1β across various cancer types (18). In addition, anticancer treatments can stimulate IL-1β production by cancer or immune cells, leading to contrasting outcomes on cancer progression (19). Similar to TNF-α, the level of this cytokine was also significantly increased. IL-6 is secreted by various cells, such as macrophages, T-cells, and stromal cells, in reaction to stimulation by TNF-α and IL-1 (20). Unlike our research, where there was no statistically significant change in IL-6 levels when exposed to ALCAR, in Baci et al.'s research on prostate cancer cells, this cytokine was reported to be reduced (5).

It seems that all the factors have ultimately contributed to the reduction in the survival of renal cancer cells.

CONCLUSION

In conclusion, our findings showed that ALCAR decreases the viability of ACHN cells in a time- and dose-dependent manner, consistent with reports of its anti-metastatic, cytotoxic, and metabolicmodulating effects. Interestingly, in our study, the levels of inflammatory cytokines such as TNF-α and IL-1β increased following ALCAR treatment, contrasts with some earlier reports. This difference may be due to variations in experimental conditions or the type of cancer being studied. Overall, it appears that all the factors examined in this study contributed in some way to reducing the survival of renal cancer cells.

REFERENCES

1. Bahadoram S, Davoodi M, Hassanzadeh S, Bahadoram M, Barahman M, Mafakher L. Renal cell carcinoma: an overview of the epidemiology, diagnosis, and treatment. G Ital Nefrol. 2022;39(3).
2. Makino T, Kadomoto S, Izumi K, Mizokami A. Epidemiology and Prevention of Renal Cell Carcinoma. Cancers. 2022;14(16):4059.
3. Jones LL, McDonald DA, Borum PR. Acylcarnitines: role in brain. Prog Lipid Res. 2010;49(1):61-75.
4. Albogami S. The Potential Inhibitory Role of Acetyl-L-Carnitine on Proliferation, Migration, and Gene Expression in HepG2 and HT29 Human Adenocarcinoma Cell Lines. Curr Issues Mol Biol. 2023;45(3):2393-408.
5. Baci D, Bruno A, Cascini C, Gallazzi M, Mortara L, Sessa F, et al. Acetyl-L-Carnitine downregulates invasion (CXCR4/CXCL12, MMP-9) and angiogenesis (VEGF, CXCL8) pathways in prostate cancer cells: rationale for prevention and interception strategies. Journal of Experimental & Clinical Cancer Research. 2019;38(1):464.
6. Ghareeb OA, Abdulla GM, Sheikhzadeh S. Evaluation of proinflammatory cytokines in arsenic induced hepatotoxicity in vivo. Journal of Animal Health and Production. 2024;12(s1):196-201.

7. Lan T, Chen L, Wei X. Inflammatory Cytokines in Cancer: Comprehensive Understanding and Clinical Progress in Gene Therapy. Cells. 2021;10(1).
8. Ghareeb OA, Sheikhzadeh S. Single Dose of Chloroform Induces Hepatic Dysfunction with Pro-Inflammatory Cytokines Response in Vivo. South Eastern European Journal of Public Health. 2024:747-53.
9. Tahmasebi S, Alimohammadi M, Khorasani S, Rezaei N. Pro-tumorigenic and Anti-tumorigenic Roles of Pro-inflammatory Cytokines in Cancer. In: Rezaei N, editor. Handbook of Cancer and Immunology. Cham: Springer International Publishing; 2022. p. 1-25.
10. Brodaczewska KK, Szczylik C, Fiedorowicz M, Porta C, Czarnecka AM. Choosing the right cell line for renal cell cancer research. Molecular Cancer. 2016;15(1):83.
11. Sarzi-Puttini P, Giorgi V, Di Lascio S, Fornasari D. Acetyl-L-carnitine in chronic pain: A narrative review. Pharmacological Research. 2021;173:105874.
12. Donisi I, Balestrieri A, Del Vecchio V, Bifulco G, Balestrieri ML, Campanile G, D'Onofrio N. l-Carnitine and Acetyl-lCarnitine Induce Metabolism Alteration and Mitophagy-Related Cell Death in Colorectal Cancer Cells. Nutrients. 2025;17(6):1010.
13. Pisano C, Vesci L, Milazzo FM, Guglielmi MB, Foderà R, Barbarino M, et al. Metabolic approach to the enhancement of antitumor effect of chemotherapy: a key role of acetyl-L-carnitine. Clin Cancer Res. 2010;16(15):3944-53.
14. Kouttab NM, De Simone C. Modulation of cytokine production by carnitine. Mediators of Inflammation. 1993;2(7):862181.
15. Haghighatdoost F, Jabbari M, Hariri M. The effect of L-carnitine on inflammatory mediators: a systematic review and metaanalysis of randomized clinical trials. European Journal of Clinical Pharmacology. 2019;75(8):1037-46.
16. Rastgoo S, Fateh ST, Nikbaf-Shandiz M, Rasaei N, Aali Y, Zamani M, et al. The effects of L-carnitine supplementation on inflammatory and anti-inflammatory markers in adults: a systematic review and dose–response meta-analysis. Inflammopharmacology. 2023;31(5):2173-99.
17. Wang S, Xu J, Zheng J, Zhang X, Shao J, Zhao L, Hao J. Anti-Inflammatory and Antioxidant Effects of Acetyl-L-Carnitine on Atherosclerotic Rats. Med Sci Monit. 2020;26:e920250.
18. Rébé C, Ghiringhelli F. Interleukin-1β and Cancer. Cancers (Basel). 2020;12(7).
19. Baker KJ, Houston A, Brint E. IL-1 Family Members in Cancer; Two Sides to Every Story. Frontiers in Immunology. 2019;10.
20 . Heikkilä K, Ebrahim S, Lawlor DA. Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. European Journal of Cancer. 2008;44(7):937-45.