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Therapeutic Impact of Fluvastatin and Metformin Against Hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the most detrimental and intrusive state of liver cancer. The incidence of HCC resumed advancing rapidly, ranking as the sixth most common cancer and the fourth ultimate cause of death. To evaluate the utility of metformin and fluvastatin, alone or in combination, as potential anticancer therapeutics using human liver cell lines (HepG2). Four groups were allocated and treated for 24 hours as follows: The control(Group1) consists of HepG2 cells treated with 0.1% dimethyl sulfoxide with an average absorption at 570 nm of 0.651; the fluvastatin (Group 2)in (6.5, 12.5, 25, 50, and 100 µM) concentrations; the metformin (Group 3) in (6.5, 12.5, 25, 50, and 100 mM); and the (Group 4) of 10 mM metformin with fluvastatin in (10, 15, 20, and 40 µM) of HepG2 cell. The results revealed a synergistic effect between metformin and fluvastatin, where the combination is more effective in achieving an IC50 of about 25. These are more potent at reducing cell viability by about 44.62%. Also, combining of 10 µM metformin with 10 and 40 µM fluvastatin concentrations. A current study demonstrated that metformin and fluvastatin separately reduced the viability of HepG2 liver cancer cells in a dose-dependent way. Also, when metformin (10 mM) was used together with fluvastatin (10–40 µM), it displayed stronger cancer-killing properties than using any drug by itself. These recommend a potential synergistic effect of the combination.

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Keywords: Fluvastatin, Metformin, Cytotoxicity, Cell Lines, Hepatocellular Carcinoma.

INTRODUCTION

Most cancers advance from chronic manifestations, and in this context, originally specified medications against the chronic disorder, such as statin and metformin, might be frequently administered during cancer therapy[1]. According to the World Health Organization, the 4th ultimate reason for death in upper-middle-income nations by 2019 is predicted to extend to 21.7 million unique cancer issues and 13 million cancer deaths by 2030[2,3]. The consequences of liver cancer coupled with the hepatitis C virus, are elevated plasma glucose concentration, hepatic steatosis conditions, adiposity, and dyslipidaemia[4,5]. Hepatocellular carcinoma (HCC) has been categorized as accounting for roughly 80% of all diagnoses[6]. In 1992, cancer cells generated an alternative means of utilizing cholesterol for cell synthesis, leading scientists to suggest that pharmaceuticals involving cholesterol utilization can control or restrict cancer growth[7]. The incidence of cirrhosis is a substantial hazard element for hepatocellular carcinoma, rendered primarily by hepatitis virus infection and alcohol misuse merged with metabolic ailments[8] serious in the elderly and male[9,10]. Different drugs have miscellaneous effects on the proliferation of cancer cells[11]. Clinical investigations have exhibited that the incidence of hepatocellular carcinomas is declined by statins[12,13]. Statin is linked to a lower risk of cancer appearance, decreases angiogenesis and metastasis, stimulates apoptosis, and guides an antitumor impact[14-17]. The mevalonate pathway was the foremost antitumor mechanism of statins that was found and is even the most exhaustively represented[18]. Statins handle Yes-associated protein (YAP) access into the nucleus by affecting the conformation of the cytoskeleton[19]. Although statins like fluvastatin (FLV) and metformin (MET) are widely assumed to be hypolipidemic and hypoglycaemic drugs, respectively, there are multiple prognoses around their improving antitumor effects when they are integrated with conventional chemotherapeutics[20]. The use of metformin or statins had a decreased risk of colon cancer compared to non-users[21]. Metformin has an acceptable safety profile with rare adverse effects, making it an engaging prospect for use in cancer medicines in vivo and in vitro[22-24]. Metformin reduced cell viability, proliferation, and migration, controlled the cell cycle, and improved the apoptosis of human cervical cancer[25].

Statins, or 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), were discovered in the 1970s by a Japanese researcher[26]. Fungal concluded statins entail lovastatin, pravastatin, and simvastatin, while nonnatural statins carry atorvastatin, cerivastatin, fluvastatin, pravastatin, pita vastatin, and rosuvastatin[27-30]. In line with medical studies, fluvastatin minimizes the chance of proliferation of HCC, prostate carcinoma, and breast cancer[12,13,28]. The use of statin decreases hepatic portal vessel pressure, less HCC prevalence, and reduced mortality in liver cirrhosis cases, with no discernible impact on the risk of bleeding or bacterial peritonitis[29]. Moreover, fluvastatin is metabolized by the CYP2C9 isoenzyme[31]. Excluding the precluding of cardiovascular disease, statins have medicinal effects in ovary and uterus-related diseases[32]. Fluvastatin reduces breast tumour proliferation, boosts apoptosis, and is associated with advantageous results on tumour cell apoptosis[33]. Fluvastatin has anti-inflammatory actions and inhibits the liberation of cytokines in diverse cell culture experiments[34] and it steadily rises cytokine creation in IL-33-refreshed mouse and human mast cells[35].

Metformin is a dimethyl biguanide, a manufactured product of glargine (isoprenyl guanidine) that originates from *Galego officinalis*[36,37]. Metformin is a widely used, first-line oral hypoglycaemic mechanism for patients with type 2 Diabetes[38]. Numerous studies have suggested that the anticancer effects of metformin in HCC can be enhanced using additional medications[39], dichloroacetate[40], loin[41], and empagliflozin[42]. Some analyses have pointed out that the defensive effect of metformin may have direct and indirect mechanisms[43]. Among the indirect mechanisms of inhibition of carcinogenic, the antitumor action of metformin attributed to both metabolic and epigenetic modulation of the cancer cells, the involvement of several signalling tracks as AMPK-dependent and AMPK-independent mechanisms[44], AKT, the mechanistic target of rapamycin (mTOR), and the activation of P53[45,20]. Metformin is collected in cells, mainly in the mitochondria[43] as a positive charge (NH3+) carries it towards the negative side of the transmembrane possibility. Metformin reduces the proliferation of hepatic triglycerides in non-alcoholic fatty liver disease and represses obesity-induced inflammation[46,47]. The combination therapy with metformin and locoregional treatment has enhanced prevalent survival, decreased tumour duplication, and declined cell proliferation and migration[48]. Metformin has numerous procedures, such as inhibition of hepatic stellate cell activation, decrease of liver fibrosis, and suppression of decompensated cirrhosis[49]. Numerous practices have studied statins and metformin for a spectrum of malignancies, including brain tumours and glioblastoma cell cultures[50-60]. The aim of this study is assess the value of metformin and fluvastatin, alone or in combination, as prospective anticancer therapeutics using human liver cell lines.

I. METHODS AND MATERIAL

As a means of our indicated objectives, we categorized the groups as follows: 0.1% DMSO-treated HepG2 cells constitute the control group (untreated sample)[52]. Fluvastatin group; HepG2 cells were treated with fluvastatin alone at concentrations of (6.5, 12.5, 25, 50, and 100 µM). Metformin group; HepG2 cells were treated with metformin alone at concentrations of (6.5,12.5, 25,50 and 100 mM). Metformin and Fluvastatin group; HepG2 cells were treated for 24 hours with 10 mM Metformin and Fluvastatin (10,15, 20, 40 µM).

Cell line and culture

Hepatocellular carcinoma cell line (HepG2) cells are generally used in pharmaco-toxicological analysis [53]. The National Cell Bank of Iran (Pasteur Institute, Iran) donated the HepG2. 10% FBS (Gibco) along with antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) for cultivating cells in RPMI-1640 (Gibco). Trypsin/EDTA (Gibco) and a phosphate-buffered saline (PBS) solution were applied to pass the cells, which were possessed at 37°C in humidified air through 5% CO_2.

MTT Assay

The MTT assay is presently the most broadly used approach for IC50 proportions. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Sigma-Aldrich) analysis was carried out to assess cell growth and viability[59]. To describe it, cells were trypsin-outlined, collected, and adapted to a density of 1×10^4 cells/ well before being planted on 96-well plates with 200 µl of fresh media per well for 24 hours. Following the formation of a monolayer, cells were revealed to 100–6.25 mM metformin (Sigma, cat. #317240) or 100–6.25 µM fluvastatin (Sigma, cat. #SML0038) for 24 hours at 37°C in 5% CO_2. The monolayer culture was kept intact in the initial plate at the final stage of the treatment (24 hours); while the supernatant was discarded, and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added. The plate was subsequently incubated for a further four hours at 37°C. The MTT solution (100 µl per well) was made by discarding the cell supernatant and putting in dimethyl sulfoxide. Cells were shaken at 37°C until the crystals melted totally. Using an ELISA reader (Model Wave XS2, Biotech, USA), absorbance at 570 nm was used to assess cell viability. The corresponding dose-response curves were consulted to calculate the concentration of the chemicals that led to 50% of cell death (IC50).

Statistical analysis

The practical results are proposed as ± the standard deviation (SD). Viability percentages were analysed using one-way ANOVA followed by using the Tukey's HSD post-hoc test using SPSS v.11 software. $P < 0.05$ was considered statistically significant.

II. RESULTS AND DISCUSSION

The inhibitory concentration (IC50) is crucial to the performance of the pharmacological and biological features of a chemotherapeutic mechanism[54]. This study concentrates on widely advised metformin (met) and fluvastatin (FLV) as anticancer activities in HPG2 cells. Various FLV and metformin concentrations were applied to determine the antiproliferative impact on HepG2 cells. IC50 values for the treatment groups were resolved as follows: FLV = 42.02663 µM, met = 45.74343 µM, and 10 mM MET + FLV = 25.22704 µM . The IC50 of fluvastatin only in HepG2 cells is 42.02 µM after 24 h; this ratio negligibly decreased cancer cell proliferation compared with combined treatments. The IC50 of metformin only in HepG2 cells is 45.74 µM after 24 h; this ratio dramatically decreased cancer cell proliferation but was less if compared with combined therapies. The IC50 of metformin and fluvastatin in HepG2 cells is 25.22 µM after 24 h; this integrated treatment in this ratio dramatically reduced cancer cell proliferation compared with single treatments. (Table I) demonstrated that the viability of HepG2 cells at 6.25, 12.5, 25, 50, and 100 µM concentrations of fluvastatin was 78.26, 69.35, 58.29, 53.99, and 44.62%, respectively. The viability of the HepG2 cells is also calculated to be at 6.25, 12.5, 25, 50, and 100 mM concentrations of metformin (83.08, 68.03, 51.64, 51.64, and 40.16%, respectively) (Table II). Then, in a combination of 10 mM metformin with 10, 15, 20, and 40 µM fluvastatin, hepG2 cell viability% is (66.44, 59.90, 48.75, and 31.11%, respectively) (Table 3). In the present investigation, the increase of hepatocellular carcinoma cells (HepG2) was restricted by fluvastatin and metformin, as demonstrated by MTT assay marks.

TABLE I: The viability measurements at different fluvastatin (FLV) concentrations.

Sample ID	(Group 2)									
Concentration FLV (µM)	6.25		12.5		25		50		100	
absorption at 570 nm	0.529	0.490	0.472	0.431	0.415	0.344	0.344	0.359	0.205	0.200
Viability (%)	81.26	75.27	72.50	66.21	63.75	52.84	52.84	55.15	31.49	30.72
Average Viability (%)	78.26		69.35		58.29		53.99		44.62	
IC50	42.0266µM									

This table illustrates the inhibitory effect of Fluvastatin (FLV) on human hepatocellular carcinoma cell lines (HepG2), the variability percentages are significant for all concentrations. Lower absorption values at 570 nm correspond to lower viability percentages, the highest average absorption is observed at 6.25 µM FLV. The data are presented as mean ± SD; $p < 0.05$ was considered significant.

* Control (Group 1) untreated sample, average absorption at 570 nm 0.671

TABLE II: The viability measurements at different Metformin concentrations.

Sample ID	(Group3)									
metformin Concentration (mM)	6.25		12.5		25		50		100	
absorption at 570 nm	0.573	0.542	0.481	0.432	0.335	0.358	0.302	0.391	0.258	0.281
Viability (%)	85.39	80.77	71.68	64.38	49.93	53.35	45.01	58.27	38.45	41.88
Average Viability (%)	83.08		68.03		51.64		51.64		40.16	
IC50	45.7434 µM									

This table illustrates the inhibitory effect of Metformin (met) on human hepatocellular carcinoma cell lines (HepG2), the variability percentages are significant for all concentrations. The highest average absorption is observed at 6.25 mM metformin. The data are presented as mean ± SD; $p < 0.05$ was considered significant.

TABLE III: The viability measurements at different fluvastatin + 10mM Metformin concentrations

Sample ID	(Group4)							
10 met (mM)+FLV Concentration (µM)	10		15		20		40	
absorption at 570 nm	0.331	0.360	0.310	0.313	0.286	0.221	0.253	0.211
Viability (%)	63.65	69.23	59.62	60.19	55.00	42.50	48.65	40.58
Average Viability (%)	66.44		59.90		48.75		31.11*	
IC50	IC50 25.227µM							

This table illustrates the inhibitory effect of 10 mM metformin(met) and fluvastatin (FLV) in proliferation of human hepatocellular carcinoma cell lines (HepG2). The data are presented as mean ± SD; $p < 0.05$ was considered significant.

Based on the abovementioned upshots, the data implies that the combination of 10 mM metformin with rising concentrations of FLV results in a dose-dependent reduction in cell viability. More elevated concentrations of FLV lead to lower cell viability, as predicted by the decreasing average viability percentages. Our results are an asset and augment previous data[55] revealing the antitumor actions of metformin, fluvastatin, and especially their combination as a possible new therapeutic mechanism for the management of HPG2.

Thus, based on all these results documented herein, it appears appropriate to indicate that the co-administration of both drugs, metformin and fluvastatin, might also exert antitumor actions in HPG2 cells[60]. It could imply that HPG2's anti-cancer effects are obtained with modest dosages or short-term metformin use. The results indicate that neither FLV nor metformin increases the incidence of HPG2. Furthermore, the increased cytotoxicity of FLV due to conjugation with metformin highlights a possible function for HPG2. Numeral of possible cells was dose-dependently decreased in metformin treated cells when compared to untreated cells [56]. The acquired results displayed that with an increase in metformin concentration, the rate of cell survival slowly decreases[57]. The potential use of fluvastatin in future immune therapy plans for combinational treatment is established in compliance with clinical tests[1]. Statins can stop cancer, especially HCC, via their anti-inflammatory and antioncogenic effects[58]. The present data reveal that metformin and fluvastatin, alone or in combination, exerted a high antitumor effect on HepG2 with significant differences observed at higher doses. The treatment of HepG2 cells with combined low quantities of FLV that significantly did not inhibit cell proliferation each (6.25 µm) resulted in statistically significant inhibition of cell proliferation (78.26% by MTT test) (Table I).

III. CONCLUSION

Typical medications, such as fluvastatin and metformin, are inexpensive, have a favourable safety profile, and could have metabolic results in additional organs. Our results display that FLV holds a dose-dependent cytotoxic effect on cells, with noteworthy reductions in viability marked at higher engagements. Similarly, metformin also exposed cytotoxic action, though its result was relatively minor . Finally, prospective reflections should concentrate on fluvastatin efficacy in other types of cytotoxicity.

IV. Ethical clearance

The study was approved by the ethical committee of the National Cell Bank of Iran (NCBI) of the Tehran Research Center (ethical code: 1000.000) in January of 2025.

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