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Evaluation of combined use of hsp90 inhibitor mpc-3100 and traditional cancer drug 5-fu on liver cancer cell lines

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Abstract

Hepatocellular carcinoma (HCC), which constitutes an important part of the global cancer burden, poses an important problem in the field of medicine. Combination therapy targets multiple mechanisms simultaneously using different therapeutic agents together. Heat shock protein 90 (HSP90) inhibitors are emerging as interesting targets in this area, since they play a vital role in the control of cellular processes and impact malignant cell survival and resistance mechanisms. This study evaluated the combined effect of the HSP90 inhibitor MPC-3100 and the traditional chemotherapy drug 5-fluorouracil (5-FU) on HCC. MTT assay was performed to evaluate the individual and combined cytotoxic effects of 5-FU and MPC-3100 on HUH-7 and HepG2 liver cancer cell lines. To assess the effectiveness of combination therapy, the Chou and Talalay method was applied. Both 5-FU and MPC-3100 and 5-FU+ MPC-3100 exhibited dose- and time-dependent cytotoxic effects. Combined administration of the two drugs showed an antagonistic impact on the cell lines. The findings demonstrated that combining 5-FU with MPC-3100 was less effective in inducing cytotoxicity in liver cancer cell lines compared to the use of each drug separately. In this context, the combination of these two drugs in liver cancer is not an appropriate strategy for effective treatment. Current research findings will help design more effective and targeted therapies for HCC and other cancers.

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Keywords: MPC-3100, 5-fluorouracil, Combination therapy, Antagonistic effect

1. Introduction

Hepatocellular carcinoma (HCC) is a significant cause of cancer mortality globally [1]. Drug resistance and adverse effects interfere with HCC therapy. Current treatment approaches have a low chance of survival and a high rate of cancer recurrence [2]. The complex molecular and cellular mechanisms in the development of HCC reveal

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the need for new therapeutic strategies to replace traditional approaches [3]. In this context, the concept of combination therapy has emerged as an effective way to achieve enhanced therapeutic efficacy in HCC. This strategic approach simultaneously targets multiple pathways and aspects of cancer formation by exploiting the potential synergy between different agents. Thus, the combined therapy approach increases the likelihood of better treatment outcomes [4]. The role of HSP90 inhibitors in such synergistic approaches is of great interest. HSP90, the master regulator of protein folding, stabilization and conformational maturation, has emerged as a very important protein in cancer biology [5]. The multifaceted role of HSP90 in governing the fate of various client proteins is crucial in cancer, with implications for growth, survival, and the development of drug resistance. Because of many aberrantly regulated proteins frequently in HCC, HSP90 stands out as a viable therapeutic target [6]. Recently, HSP90 was found as a potential biomarker of HCC, and its expression has proven to have a major diagnostic value for HCC diagnosis [7]. Inhibition of HSP90 becomes an attractive therapeutic target in the context of HCC because it offers the opportunity to affect multiple oncogenic signaling pathways simultaneously. In this study, MPC-3100 was chosen as a promising candidate due to its excellent pharmacological properties [8]. MPC-3100 inhibits ATPase function by targeting Hsp90 N-terminal ATP binding domain. MPC-3100 was shown to inhibit HSP90 activity in luciferase degradation studies with an IC_{50} value of 60nM. MPC-3100 inhibited cell growth in the HCT-116 colon cancer cell line with an IC_{50} value of 540nM. These findings highlighted ability of MPC-3100 to disrupt essential cancer development pathways. MPC-3100 was also found to have broad-spectrum anti-proliferative effect against the NCI-N87 gastric cancer and DU-145 prostate cancer cell lines [8]. MPC-3100 has been demonstrated to decrease tumor development in the NCI-N87 gastric cancer xenograft model. MPC-3100 has a high oral pharmacokinetic profile, excellent overall exposure, and an acceptable hepatic clearance rate, according to pharmacokinetic studies [9]. MPC-3100 was well tolerated in the Phase I human clinical study and displayed pharmacokinetic and pharmacodynamic characteristics comparable to those shown in preclinical research [10]. These pharmacological properties make MPC-3100 stand out as an ideal possibility for further research, especially in the context of combination therapy with conventional chemotherapeutic drugs.

5-fluorouracil (5-FU) is an essential antimetabolite and cell cycle inhibitor. This drug has been frequently used to treat stomach, colorectal, and breast malignancies [11]. 5-FU works against tumors by inhibiting thymidylate synthase and RNA/DNA processing. 5-FU has been studied alone and in combination in many cancer cell lines. In these studies, the IC_{50} values of 5-FU ranged between 2.3-13.0 μ M in breast cancer cells [12] and 21.9-43.2 μ mol/L in hepatocellular carcinoma cell lines [13]. The FOLFOX4 regimen using 5-FU has shown to be advantageous in the treatment of advanced HCC [14]. The FOLFOX4 regimen encompasses a combination of oxaliplatin, 5-FU and leucovorin, constituting a conventional chemotherapeutic regimen in the treatment of some advanced cancer [15, 16]. This therapy method has improved the overall survival of HCC patients. However, due to the drug's limited efficacy, it may be necessary to use higher doses of 5-FU, which can cause leukopenia, nausea, vomiting and skin rashes [17]. Consequently, It is necessary to find more effective strategies to increase the sensitivity of HCC to chemotherapy and reduce side effects [18].

The objective of this research was to examine at the possible effects of combining the standard chemotherapeutic drug 5-FU with the HSP90 inhibitor MPC-3100. The cytotoxic effect of agents alone and in combination on two liver cancer cell lines were evaluated at 24h and 48h. To assess drugs interactions, the Chou and Talalay method was employed.

2. Materials and Methods

2.1. Chemicals and Reagents

3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Serva. American Type Culture Collection provided the HUH-7 and HepG2 cell lines. Trypsin-EDTA, penicillin-streptomycin solution, Dulbecco's Modified Eagle's medium (DMEM) High Glucose, L-glutamine, fetal bovine serum (FBS) and

phosphate buffer saline (PBS) were supplied from Biological Industries. MPC-3100 was obtained by AdooQ® Bioscience. 5-FU was purchased from Gold Biotechnology.

2.2. Cell Culture

The cell lines HUH-7 and HepG2 were cultivated in DMEM High Glucose medium containing 10% FBS, 0.1% penicillin-streptomycin and 1% L-glutamine. The cell lines were meticulously maintained under controlled conditions in a humidified environment at 37 °C with 5% CO₂.

2.3. Cell Viability Assay

The cytotoxic effects of MPC-3100 and 5-FU were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. This method relies on the principle of MTT conversion, wherein viable cells transform MTT into insoluble formazan crystals through mitochondrial dehydrogenase activity. These crystals are subsequently dissolved in dimethyl sulfoxide (DMSO) and their absorbance at 570 nm wavelength is measured using a spectrophotometer [19]. The cancer cells were meticulously seeded in 96-well culture plates at a density of 5×10^4 cells per well and incubated at 37°C with 5% CO₂ for 24 h. After this period, the culture medium was removed from the cells. MPC-3100 and 5-FU were individually dissolved in DMSO. The stock solutions for both drugs were at a concentration of 100 mM. The treatment of the cancer cell lines with the drugs was performed by diluting them from the DMSO stock solution into DMEM High Glucose medium. DMSO is non-toxic to HUH-7 and HepG2 cell lines when the final concentration is 0.1% [20]. In this study, DMSO solvent was used at a final concentration below 0.1%. The cells were treated with MPC-3100 and 5-FU at concentrations ranging from 5 μM to 0.078 μM and 10 μM to 0.156 μM for 24h and 48h, respectively. Individual drug cytotoxicity experiments were performed in serial dilutions, with maximal initial concentrations of 5 μM for MPC-3100 and 10 μM for 5-FU. Taking into account cytotoxicity values of the drugs, the ratio of 5 μM: 5 μM, i.e. 1:1, was investigated in combination tests and this ratio was kept constant. As a control group, cells were not treated with drugs and only DMEM high glucose medium was added. Following incubation, the medium was removed, and 100 μL of MTT solution (prepared fresh by adding 1 mL of 5 mg/mL MTT solution in PBS to 9 mL of medium) was added to each well. The cells were incubated at 37°C for 3 h. Following this, the MTT solution on the cells was aspirated and the resulting formazan crystals were dissolved by adding 100 μL of DMSO. Subsequently, the absorbance was then measured at wavelength of 570 nm using a spectrophotometer [21]. The following formula was used to determine cell viability as a percentage relative to the control group:

$$\% \text{ Cell viability} = (\text{Absorbance of experimental group} / \text{Absorbance of control}) \times 100.$$

Nonlinear regression analysis was performed to determine the half-maximal inhibitory concentration (IC₅₀) of each drug, and the IC₅₀ value was determined with a sigmoidal dose-response curve using GraphPad Prism 8.0.2 software.

2.4. Statistical Analysis

Data analysis and comparison were conducted using GraphPad Prism 8.0 software, with significance set at $p < 0.05$. The potential synergistic effect of the drug combination was assessed using the Chou and Talalay method, and the combination index (CI) was calculated utilizing CompuSyn software.

3. Results and Discussion

HUH-7 and HepG2 cells were used to investigate the combined effect of 5-FU and MPC-3100 on liver cancer. HUH-7 and HepG2 cell lines are widely acknowledged as the gold standard and are renowned for faithfully representing the characteristics observed in liver cancer [22]. These cell lines were selected for our investigation because they have a well-established reputation for correctly reflecting the characteristics of liver cancer. HUH-7 and HepG2 cells are epithelial in origin. Gene expression differs between the two cell lines [23]. A known difference between different HepG2 and HUH-7 cells is the different p53 status. HepG2 cells have wild-type p53 expression, whereas HUH-7 cells have mutant-type p53 expression [24]. HSP90's main client protein is p53, which is mutated in more than half of all human malignancies. Previous research has demonstrated that inhibiting HSP90 can cause mutant p53 to be ubiquitinated and degraded via the proteasome way. Because functioning p53 is present, inhibiting HSP90 activity in this manner might cause apoptosis [25]. In conclusion, we found that combining MPC-3100 with 5-FU might activate various pathways in HepG2 and HUH-7 cells. The MTT experiment was used to investigate the effect of 5-FU and MPC-3100 on cell viability after 24 and 48 hours as a single agent and in combination. As shown in Fig. 1, 5-FU and MPC-3100 inhibited cell viability in HepG2 and HUH-7 cells in a time- and dose-dependent manner.

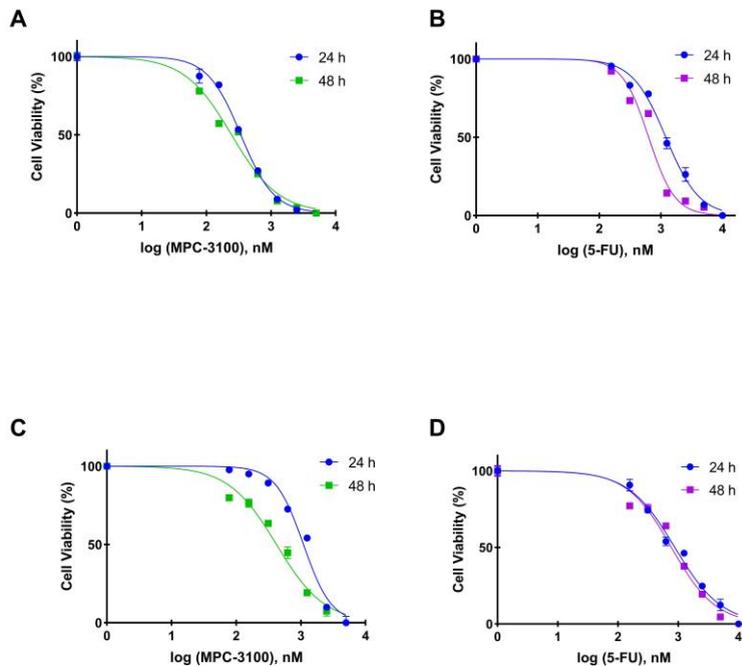


Fig 1. Antiproliferative effect of MPC-3100 (A), 5-FU (B) in HepG2 cell line and MPC-3100 (C), 5-FU(D) in HUH-7 at 24 h and 48 h.

Considering the IC_{50} values given in Table 1, HSP90 inhibitor MPC-3100 inhibited cell growth at low concentrations in both HepG2 and HUH-7 cell lines. Previous research has found that inhibiting HSP90 causes cell death in liver cancer cells. Watanabe et al. showed that treatment of HCC cells with the HSP90 inhibitor 17-AAG resulted in decreased viability of HCC cells and apoptosis of the cells. In addition, 17-AAG treatment caused an increase in the ratio of cells in the G2/M phase and decreased cdc2 protein degradation [26]. Compared to SNX-2112 17-AAG, an HSP90 inhibitor, showed a high inhibition effect on cell growth and triggered caspase-related apoptosis in these cells [27]. Administration of 17-DMAG HCC cells showed a cytotoxic effect on cells by decreasing NF- κ B, cyclin D1 and survivin protein levels and increasing p53 protein levels [28]. When cells were treated with DMAG-N-oxide, the mean number of migrating cells was dramatically reduced [29].

Dose-dependent cell death occurred in both HepG2 and HUH-7 cells treated with 5-FU at 24h and 48h, and 5-FU showed a lower concentration of cytotoxic effect on HepG2 cells at 48h compared to HUH-7 cells. 5-FU is a cancer treatment drug that is frequently utilized. It produces cytotoxicity either by inhibiting the activity of the thymidylate synthase (TS) enzyme, preventing basic biosynthetic activity, or by inadvertently incorporating its metabolites into RNA and DNA [30]. However, chemotherapy resistance to 5-FU is a problem for many types of cancer, including liver cancers. When used in combination with other chemotherapies, effectiveness of 5-FU can be increased. Cisplatin combined with hepatic arterial infusion and 5-FU enhanced HCC patient survival compared to patients who did not receive the combination of treatments [31]. Clinical trials involving the combined use of cisplatin and 5-FU are being conducted in HCC patients who develop sorafenib resistance (NTC02967887) [17].

Table 1. IC₅₀ values of MPC-3100 and 5-FU in HepG2 and HUH-7 cell lines at 24 h and 48 h.

Cell Lines	IC ₅₀ (nM)			
	MPC-3100		5-FU	
	24 h	48 h	24 h	48 h
HepG2	341.5 ± 0.002	245.4 ± 0.004	1203 ± 0.004	633.3 ± 0.005
HUH-7	1097 ± 0.005	419.4 ± 0.005	879.0 ± 0.004	770.6 ± 0.005

Because of multidrug resistance and dose-dependent cytotoxicity, 5-FU-based chemotherapy has poor clinical anti-cancer effectiveness. To address these issues, a new combination of 5-FU and additional anticancer drugs with different mechanisms of action is required. In this research, we investigated if the HSP90 inhibitor MPC-3100 and 5-FU could increase the anti-tumor effect on liver cancer cells. When we tested the effect of both drugs in combination on liver cancer cells, we discovered that co-administration of MPC-3100 and 5-FU had an antagonistic effect on both HepG2 and HUH-7 cell lines, which contradicted the predicted synergy commonly sought in combination (Fig. 2A and 2B). These findings indicate that the combination of the two drugs is less effective at inhibiting cell viability in liver cancer cells than alone. To confirm this antagonism, we administered the cells at a constant rate with a mixture of two drugs. The combination index (CI) was determined using the CompuSyn tool, as described in Chou and Talalay technique [32]. The Chou and Talalay method stand out as a basic approach in drug combination studies. Its foundations are based on the median effect equation. This equation is derived from the basic principle of the law of mass action, which combines single and multi-entity scenarios. The integration of the median-effect equation grounded in the mass action law has accelerated its practical applications [33, 34]. The use of the Chou and Talalay method became widespread after introduced an updated program called CompuSyn in 2005 [35]. This advanced program further streamlines the analysis process by allowing comprehensive evaluations such as dose ranges, combination ratios, design layouts, and even computerized simulations of drug interactions. The synergistic integration of CompuSyn and the Chou and Talalay method has become a powerful tool to unravel the complexity of drug combinations [36]. The study suggests that the synergistic, additional, and antagonistic effects of two agents may be characterized as follows: when CI is less than one, it represents synergistic effects; when CI equals one, it indicates additional effects; and when CI is greater than one, it indicates antagonistic effects [37]. HepG2 (Fig. 2C) and HUH-7 (Fig. 2D) liver cancer cells demonstrated antagonistic activity when MPC-3100 and 5-FU were combined.

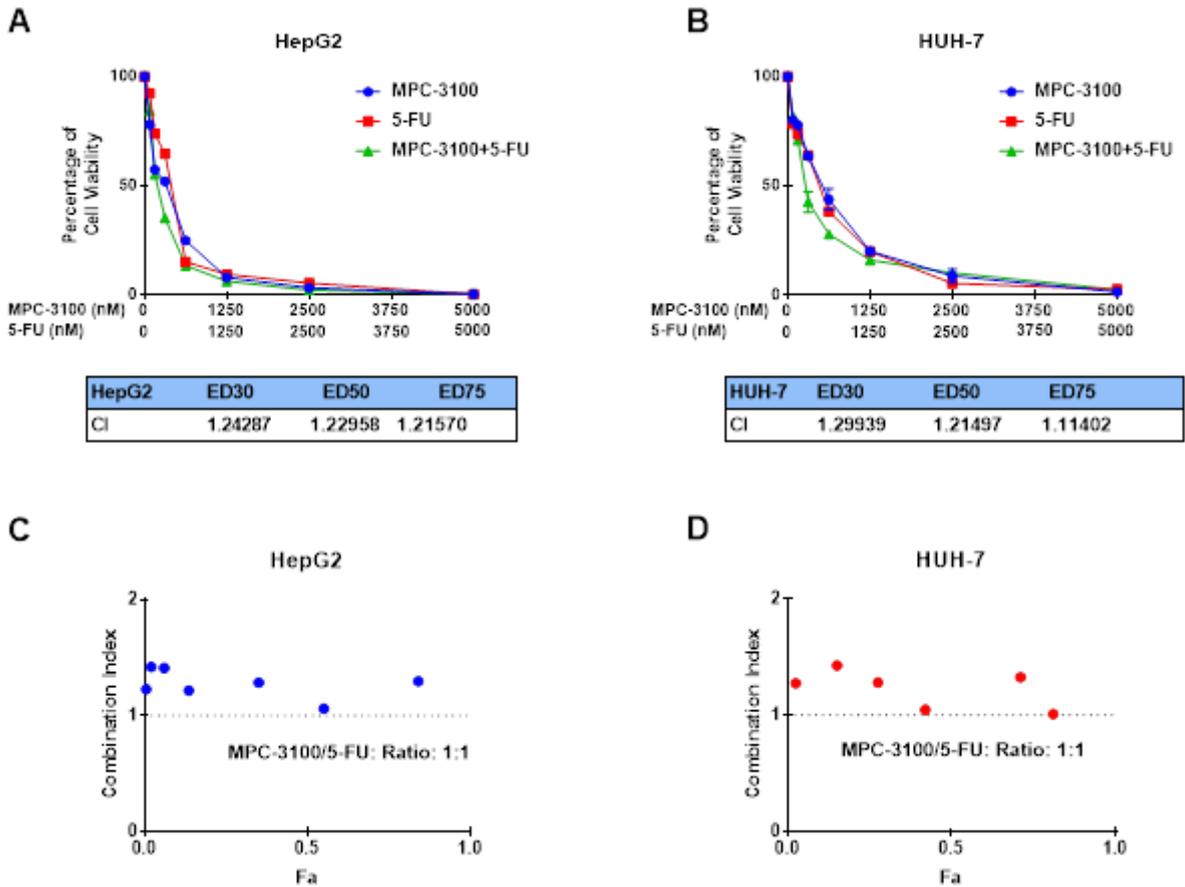


Fig. 2. Survival graphs of HepG2 (A) and HUH-7 (B) cancer cell lines treated with the combination of MPC-3100 and 5-FU; graphs were drawn from combination index data versus fraction data (Fa) from the Chou and Talalay median effects analysis for HepG2 (C) and HUH-7 (D). *ED30, ED50, and ED75 are dosages that effectively block 30%, 50%, and 75% of cells or organisms, respectively (These data were calculated using CompuSyn software.). CI, combination index Fa, fraction affected. The values give information on a drug's or treatment's potency, indicating its potential to deliver desired results at specified dose levels.

In the literature, combinations of Hsp90 inhibitors with chemotherapy drugs (eg, cisplatin in glioma, docetaxel and paclitaxel in non-small cell lung cancer, and paclitaxel and cisplatin in head and neck squamous cell carcinoma) have been shown to be synergistic in most cases [38-40]. Liu et al. investigated the effect of SNX-2112, an HSP90 inhibitor, in combination with 5-FU in esophageal cancer and revealed that the combined use of the two drugs showed antagonistic effects in cells. When the molecular mechanism of this unexpected effect was investigated in detail, several possible causes were revealed [41]. Combining SNX-2112 and 5-FU may have opposing effects by reversing G2/M arrest, preventing initial mitochondrial membrane potential reduction, reducing Hsp90 client proteins and suppressing caspase-dependent apoptosis. In our study, the reason for the antagonistic effect of the combination of HSP90 inhibitor and 5-FU in liver cells may include the suggested reasons. In conclusion, while a more precise mechanism of the antagonistic action of HSP90 inhibitor with 5-FU needs to be further demonstrated

in other types of cancer. In clinical practice, the combination of HSP90 inhibitor and 5-FU should be used with caution.

4. Conclusion

This study demonstrates the potential of combining traditional chemotherapy drug 5-FU and next-generation HSP90 inhibitor MPC-3100 as a synergistic approach to treat HCC. The experiment demonstrated the dose- and time-dependent cytotoxic effects of both drugs on the liver cancer cell lines HepG2 and HUH-7, separately. The data from the combined usage of the two drugs revealed unexpected effects. MPC-3100 and 5-FU co-administration had an antagonistic impact on both HepG2 and HUH-7, rather than the predicted additive or synergistic effects. This surprising result highlights the intricate interplay between various drugs, stressing the need of considering all factors when developing combination therapy. It is obvious that combining MPC-3100 with 5-FU may not be an effective technique for increasing cytotoxicity in liver cancer cells. However, this finding serves as a springboard for future study, stimulating searching for novel drugs combinations and techniques to addressing the complicated nature of HCC. Experiences gained from research like ours will help to design more effective and focused treatments to combat HCC and other cancers as the field advances.

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