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Synergistic Anti-proliferative Effects of Cucurbitacin I and Irinotecan on Human Colorectal Cancer Cell Lines

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Abstract

Colorectal cancer is the third most common cancer in men and the second in women worldwide. Treatments used for colorectal cancer include some combinations of chemotherapy, radiation therapy, surgery and targeted therapy to increase survival rates of cancer patients and decrease mortality caused by colorectal cancer. One of the many common chemotherapy drugs used in metastatic and recurrent colorectal cancer is the topoisomerase I inhibitor irinotecan. Combination of chemotherapy drugs is a common practice in the treatment of cancer. This study investigates the synergistic anti-proliferative effects of irinotecan with cucurbitacin I. The combination of anti-proliferative agents can potentiate the therapeutic effects, reduce the dose, and consequently, the toxicity, and minimize or delay cases of drug resistance. Cucurbitacin I is a selective Janus kinase (JAK2)/Signal Transducers and Activators of Transcription (STAT3) signaling pathway inhibitor. Activation of JAK2/STAT3 plays a crucial role in cell survival and proliferation. Thus, the identification of a compound that blocks this pathway would contribute significantly to growth inhibition and apoptosis of tumor cells. The aim of the present study was investigate the effects of cucurbitacin I and combination with irinotecan which have apoptotic anti-migratory, anti-clonogenic and antiproliferative effects on SW620 and LS174T colon cancer cell lines. In addition to this, determination of the exact molecular effects of cucurbitacins would allow us to identify new molecular targets for the treatment of the colon cancer.

Keywords: Colorectal malignancy; Cucurbitacin I; Irinotecan; JAK2/STAT3 pathway; SW620; LS174T cell lines

Introduction

Colorectal cancer is still the most common cause of cancer-based deaths in all over the world, despite of the latest improvements in the diagnosis, treatment and screening methods [1]. Tumors that are localized in the colorectal region can cause acute symptoms of obstruction that leads to the operations which have high rates of mortality and morbidity [2]. Colorectal cancer is the second most common cause of cancer death in both men and women. The incidence rates of colorectal cancer are slightly higher in men than in women [3,4]. Combination therapy with multiple drugs is a common practice in the treatment of cancer to get an additive or synergistic effect and to reduce toxicity to the host [5,6]. The most common first line chemotherapy regimen has been prescribed that FOLFOX (combination of leucovorin calcium/folinic acid, fluorouracil, oxaliplatin) 30.7% in metastatic colorectal cancer patients. Oral capecitabine has been used 15.2% [7]. Bhattacharya et al. has been reported that the most prescribed drug was capecitabine 81% [8]. Another study demonstrated that capecitabine can safely replace 5-fluorouracil in combination with oxaliplatin and irinotecan (irinotecan 180 mg/mq on day 1, oxaliplatin 85 mg/mq on day 2, capecitabine 2000 mg on days 2-6) with promising results in terms of activity [9].

The different pathways involved in the process of colorectal carcinogenesis [10]. The JAK/STAT pathway is one of the best understood signal transduction cascades [11]. Abnormalities in the JAK2/STAT3 pathway are involved in the pathogenesis of colorectal cancer, including apoptosis. Inhibition of JAK2/STAT3 signaling, induced colorectal cancer cells apoptosis [12]. Impaired in cancer cells JAK2/STAT3 signaling pathway has many inhibitors. In one of them is cucurbitacin I. In general, cucurbitacins are considered to be selective inhibitors of the JAK/STAT pathways [13,14].

Cucurbitacins are compounds isolated from various plant families, which have been used as folk medicines for centuries in countries such as India and China because of their wide spectrum of pharmacological activities such as anticancer [13-16], cytotoxic [17] and anti-inflammatory [18] effects. Of these compounds, cucurbitacin I is a natural component extracted from plants of the *Cucurbitaceae* family and has chemopreventive potential in numerous human cancer cell lines and tumor xenografts, including breast, prostate, lung, uterine cervix, liver, skin, and brain cancers [13-22]. Cucurbitacins have toxic effects as well as their biological activities. It was demonstrated that the most toxic cucurbitacins (D and I) have an unsaturated side chain and a free hydroxyl group. The main toxicological effects of cucurbitacin D and I appears to be an increase in the capillary permeability, irritate the intestinal mucosa, and strongly increase intestinal motility. The maximum tolerated dose has been determined as 0.4-0.8 mg/kg body weight for cucurbitacin I [23].

Camptothecin is a natural DNA topoisomerase I inhibitor. İrinotecan is a semi-synthetic camptothecin derivative alkaloid that has been used for the treatment of colon cancer [24].The US Food and Drug Administration (FDA) have approved irinotecan as first-

linetherapy for patients with metastatic colorectal cancer [25]. Irinotecan is converted by carboxylesterases to the active metabolite SN-38 which has 100-1000 times greater cytotoxic activity than the parent molecule. In plasma SN-38 presents about one percent of irinotecan. Carboxylesterase enzim activity for converting irinotecan into SN-38 in proliferating and live cells is higher than in low pH and hypoxic cells. The lacton ring of SN-38 is responsable for cytotoxicity. So that the cytotoxic lactone form of irinotecan is more specifically under acidic conditions than physiological or higher pH [26,27]. SN-38 glucuronidated to more polar and inactive SN-38 glucuronide by hepatic UDP-glucuronosyltransferase 1A1 (UGT1A1) and eliminated in the bile and urine [25].

Irinotecan has been used for the treatment of several cancers including lymphoma, lung, gastrointestinal and pancreatic tumours along colorectal cancers [28]. The maximum tolerated dose for irinotecan is 125 mg/m^2 when given weekly. Holcombe et al. recommended monthly cycles at dosages of 62 mg/m^2 by i.v. bolus, weekly 3, for irinotecan as an initial starting dose for colon cancer therapy [29]. Irinotecan has an acceptable tolerability profile and is not associated with cumulative toxicities in patients with metastatic colorectal cancer [30]. Even so, irinotecan has also gained attention with respect to a comparably high incidence of unpredictable severe toxicity. Neutropenia, diarrhea, nausea and vomiting are the main toxicities of irinotecan, and may result in dose reduction, treatment withdrawals or death [28,31]. Toxic effects of irinotecan such as diarrhea and leukopenia are the dose-limiting toxicities of irinotecan, which are associated with increased levels of SN-38 [25]. Some complications are observed less frequently such as asthenia and febrile neutropenia/sepsis. The safety profile of irinotecan can be improved with careful patient monitoring, routine administration of antiemetics prior to irinotecan infusion, and prompt, aggressive treatment of delayed-onset diarrhea [32].

By inhibiting topoisomerase I, an enzyme that catalyzes breakage and rejoining of DNA strands during DNA replication, SN-38 causes DNA fragmentation and programmed cell death [25-33].

To date, however, no study has been conducted in the evaluations of the combination effects of cucurbitacin I and irinotecan against human colorectal cancer cells. Therefore, the present study was designed to investigate the anti-proliferative effects of combining cucurbitacin I with irinotecan in colorectal cancer *in vitro*.

Materials and Methods

Cell culture

Human colorectal cancer cell lines SW620 and LS174T were purchased from the German Cancer Research Center (Heidelberg, Germany). The SW620 and LS174T cells were cultured in RPMI 1640 medium at 37°C in a 5% CO_2 incubator. Cells were passaged two or three times a week to maintain them in a logarithmic growth phase. For isolation and propagation, the medium was discarded, and then the cells were washed with phosphate-buffered saline (PBS), 3-5 ml trypsinized (0.25% trypsin/EDTA), pelleted at 1,500 rpm for 5 min and re-suspended at the desired concentration in RPMI-1640 medium.

Cell proliferation assay

LS174T and SW620 cells were pre-cultured in a 96-well plate (Sarstedt, Germany) overnight to allow them to adhere. Cell proliferation was assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dye reduction assay. In brief, MTT (Applichem, Germany) solution (5 mg/ml in PBS) was added (10 μl/well). Plates were further incubated for 3-4 h, and following removal of the medium, the formazan crystals were dissolved by the addition of 100 μl solvent (0.04 N HCl acid in 2-propanol) per well and then by thoroughly mixing. Optical density was measured at a 570 nm wavelength using an ELISA plate reader (BioTec, USA). MTT assay was determined by the method described by Eyol [34] with some minor modifications.

For the growth curves, LS174T and SW620 cells were seeded in 96-well microplates at final concentrations of 1×10^3, 2×10^3, 3×10^3, 4×10^3 and 5×10^3 cells/well. Cells were incubated with increasing concentrations irinotecan (1 μm, 5 μm, 25 μm, 125 μm, 250 μm) and cucurbitacin I (1, 2 nM, 6 nM, 30 nM, 150 nM, 750 nM) and combination of irinotecan with cucurbitacin I at same concentrations for 24, 48 and 72 h. Cell survival rates were expressed as the percentage of untreated controls at 24, 48 and 72 h.

In vitro wound healing assay

LS174T and SW620 cells were seeded (60,000 cells/well) in 24-well plates and allowed to attach to the surface under standard incubation conditions for 24 h. After 24 h, the confluent cell monolayers were scratched in a straight line using a 200 μl sterile plastic pipette tip, as previously described by Liang [35] with some modifications described by Kaleağasıoğlu [36]. The cells were then carefully rinsed with culture medium to remove free-floating cells and debris. Then, irinotecan and cucurbitacin I were added at final concentrations (1 μm, 5 μm, 25 μm, 125 μm, 250 μm) and (1.2 nM, 6 nM, 30 nM, 150 nM, 750 nM) respectively and combination of irinotecan with cucurbitacin I at same concentrations and the effect on wound healing was monitored. Both cell lines were studied in parallel, and the duration of the microscopic procedure was kept the same to exclude environmental condition-related differences in wound healing responses.

Clonogenic assay

This assay is an *in vitro* cell survival and proliferation assay based on the ability of a single cell to grow into a colony. Briefly, 500 cells were mixed gently and plated on a 6-well plate [37]. After being incubated for 24 h, the cells were transfected with irinotecan, cucurbitacin I and combination of irinotecan with cucurbitacin I every 5 days, and about 2 weeks later, the cells were washed with phosphate-buffered saline and stained with crystal violet. Colonies with a diameter of more than 50 cells were counted. The experiment was repeated three-times.

Statistical analysis

Data were analyzed using SPSS software ver15.0. Results of all experiments are expressed as mean values ± standard deviation SD. The Mann-Whitney U test was used to compare two groups of unpaired values, whereas for comparisons between groups of more than two unpaired values, the Kruskal-Wallis H test was used. P-values ≤ 0.05 were considered to indicate statistically significant results. ImageJ 1.48 program was used to analyze pictures of wound healing test.

Discussion

Cucurbitacins and their derivatives are triterpenoids found in medicinal plants known for their diverse pharmacological and biological activities, including anticancer effects, throughout human history [5-38]. Although initial attention to cucurbitacin as a potential anticancer drug withered for decades, recent discoveries showing that cucurbitacin is a strong STAT3 inhibitor have reclaimed the attention of the drug industry one more time [22,34]. The molecular landscape of colorectal cancer is complex. There are several oncogenic signaling pathways that are commonly involved in cancer cell proliferation and survival [10]. STAT3 activation in colorectal cancer stimulates cell proliferation, survival and tumor growth. Therefore STAT3 inhibition sensitizes colorectal cancer to chemoradiotherapy both *in vitro* and *in vivo*. In addition to JAK/STAT pathway, MEK/ERK and PI3K/AKT signaling pathways also associated with tumorigenesis and prognosis of colorectal cancer. Consequently, there are three important signaling pathways in colorectal cancer tumorigenesis and prognosis involving JAK2/STAT3, PI3K/Akt/mTOR, and RAS/MEK/ERK pathways [39]. In the present study we have shown that combination of irinotecan and cucurbitacin I has inhibitory effect on JAK2/STAT3 pathway which is one of these three pro-metastatic pathways on colorectal cancer cell lines.

Many studies confirmed that cucurbitacin I is a powerful JAK-STAT inhibitor by blocking the tyrosine phosphorylation of STAT3 and JAK2 in various human cancers [14-36]. Inhibition of STAT3 may lead to apoptosis and inhibition of tumour growth [12].

In the clinic, many anticancer drugs are used together in combinations, not only to enhance the efficacy of the treatment, but also to avoid the build-up of resistance in cancer cells [1-40]. Multidrug resistance cause treatment failure leads to undesirable toxic effects and deaths in cancer patients. As distinct from normal drug resistance, intracellular chemotherapeutic drug concentration decreases in multidrug resistance. As a result of this cancer cells are not indestructible enough [41]. Moreover, some drug combinations show strong synergism that helps to achieve the same therapeutic effect with lower dose and, hence, less toxicity [34-42]. Some reports have shown that cucurbitacins show a synergistic effect with chemotherapeutic agents that are already established in the treatment of human cancers [38-42].

The aim of the present study was to evaluate the anti-proliferative activity of cucurbitacin I and irinotecan combination in comparison with monotherapy regimens in the *in vitro* treatment of colorectal cancer cell lines. As a result of this study the viability of SW620 and LS174T cells were decreased depending on dose and time in the presence of irinotecan or cucurbitacin I alone and coadministration. Cucurbitacin I can potentiate the inhibitory effects of irinotecan on colorectal cancer cells. We demonstrate that cucurbitacin I make cancer cells more sensitive to irinotecan. In addition, the side effects of irinotecan can reduce depending on the lower dose irinotecan.

Moreover, simultaneous exposure to the combination produced greater therapeutic effect than that by single drug alone, suggesting that synergistic effect occurred on SW620 and LS174T cell lines. In conclusion, we found that combining irinotecan with cucurbitacin benefits patients with metastatic colorectal cancer. Although the studies that have investigated the effect of cucurbitacins on diffent cancer cells the number of colorectal cancer is limited. This is the first study demonstrate that treatment with cucurbitacin I alone and in combination with irinotecan decreases cell proliferation and increases apoptosis on human colorectal cancer cell lines. Cucurbitacin's synergism with already established chemotherapeutic agents is a big advantage. More studies both *in vitro* and *in vivo* on a variety of cancers will confirm the usefulness of cucurbitacin and reinvite this old drug to the modern clinic.
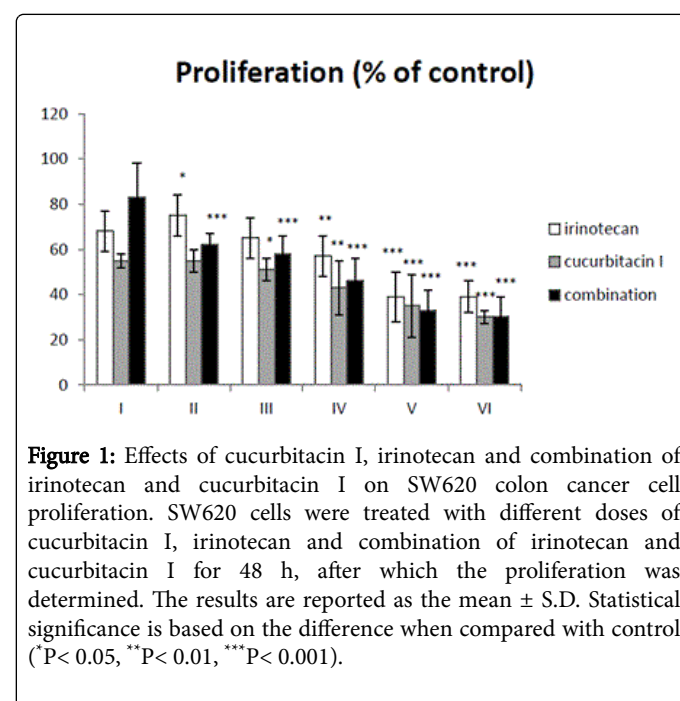
Cucurbitacins deserve future investigations targeting their discovery in uninvestigated sources and their derivatives for improving their anticancer abilities. Moreover, preclinical and clinical studies using combined treatment composed of cucurbitacins and standard chemo-, immuno and/or radio-therapies should be planned for.

Results

Cucurbitacin I and co-administration with irinotecan inhibit colon cancer cell proliferation
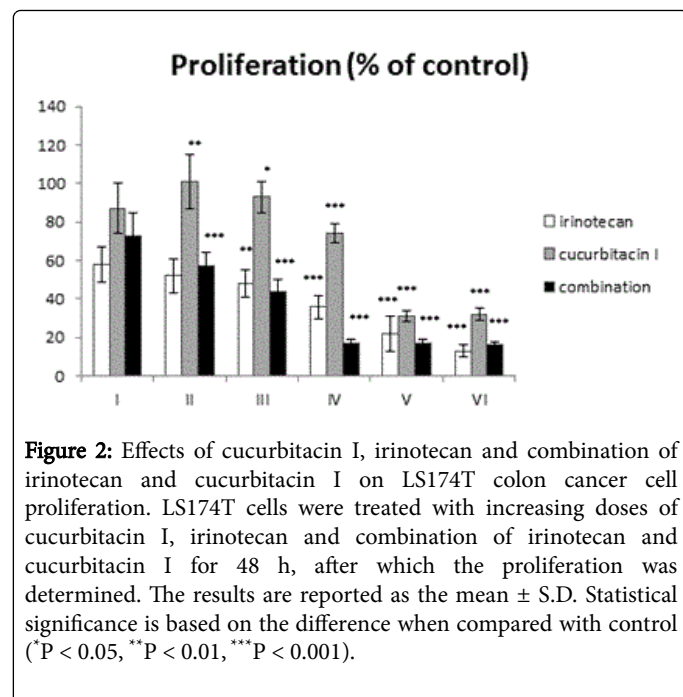
Since abnormal cell proliferation is a hallmark of cancer cells, we examined whether cucurbitacin I affected the proliferation of human colorectal cancer cells. SW620 (Figure 1) and LS174T (Figure 2) cells were treated with different concentrations (1.2, 6, 30, 150 or 750 nM) of cucurbitacin I, (1, 5, 25, 125 or 250 μm) of irinotecan and same concentrations of cucurbitacin I and irinotecan combination for 48 h.

Group I was untreated control and other groups have been set according to these increasing doses. (Group II: 1.2 nM cucurbitacin I + 1 μm irinotecan, Group III: 6 nM cucurbitacin I + 5 μm irinotecan, Group IV: 30 nM cucurbitacin I + 25 μm irinotecan, Group V: 150 nM cucurbitacin I + 125 μm irinotecan, Group VI: 750 nM cucurbitacin I + 250 μm irinotecan). Cell proliferation was assessed by the MTT assay.

Figure 1: Effects of cucurbitacin I, irinotecan and combination of irinotecan and cucurbitacin I on SW620 colon cancer cell proliferation. SW620 cells were treated with different doses of cucurbitacin I, irinotecan and combination of irinotecan and cucurbitacin I for 48 h, after which the proliferation was determined. The results are reported as the mean ± S.D. Statistical significance is based on the difference when compared with control (*P< 0.05, **P< 0.01, ***P< 0.001).

The treatment irinotecan and cucurbitacin I individually and combined was reduced cell proliferation on SW620 cells increasing doses. When cells were treated with irinotecan combined with cucurbitacin I the anti-proliferative effect was significantly increased at 150 nM + 125 μM dose.

Figure 2: Effects of cucurbitacin I, irinotecan and combination of irinotecan and cucurbitacin I on LS174T colon cancer cell proliferation. LS174T cells were treated with increasing doses of cucurbitacin I, irinotecan and combination of irinotecan and cucurbitacin I for 48 h, after which the proliferation was determined. The results are reported as the mean ± S.D. Statistical significance is based on the difference when compared with control ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

The treatment irinotecan and cucurbitacin I individually and combined was reduced cell proliferation on LS174T cells increasing doses. The results obtained suggest that irinotecan combined with cucurbitacin I induced anti-proliferative effects on LS174T cells except the 1.2 nM + 1 μm dose. As shown in Figure 2 combination of irinotecan and cucurbitacin I produced more remarkable and statistical significance inhibition of the proliferation of LS174T cells.

We have determined that a dose dependent decrease in cell viability of both SW620 and LS174T cells which treatment with cucurbitacin I and its combination with irinotecan. Based on these data we suggest that when administered separately, cucurbitacin I and irinotecan and co-administration of cucurbitacin I and irinotecan led the cells to apoptosis in SW620 and LS174T cells by blocking cell proliferation. It was observed that further increase anti-carcinogenic effect after the implementation of cucurbitacin I and irinotecan together.

Wound healing assay in SW620 and LS174T cells

The wound healing assay was followed over 48 h for the low concentrations used in the cytotoxicity assay. Wound width was expressed as μm (Figure 3).
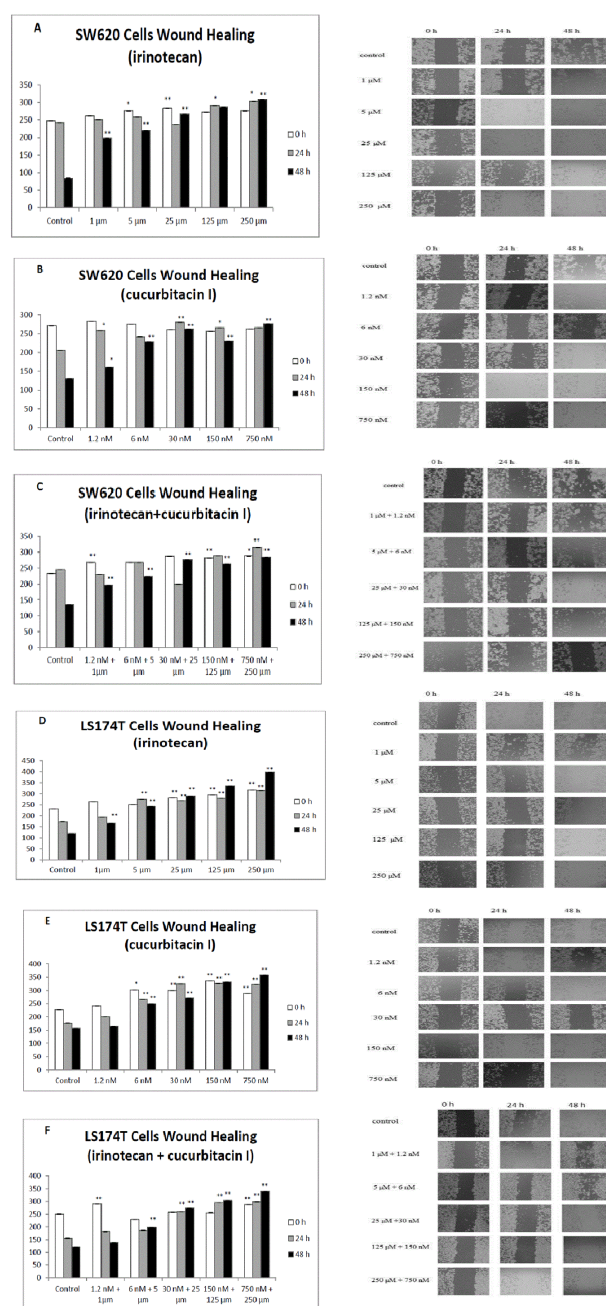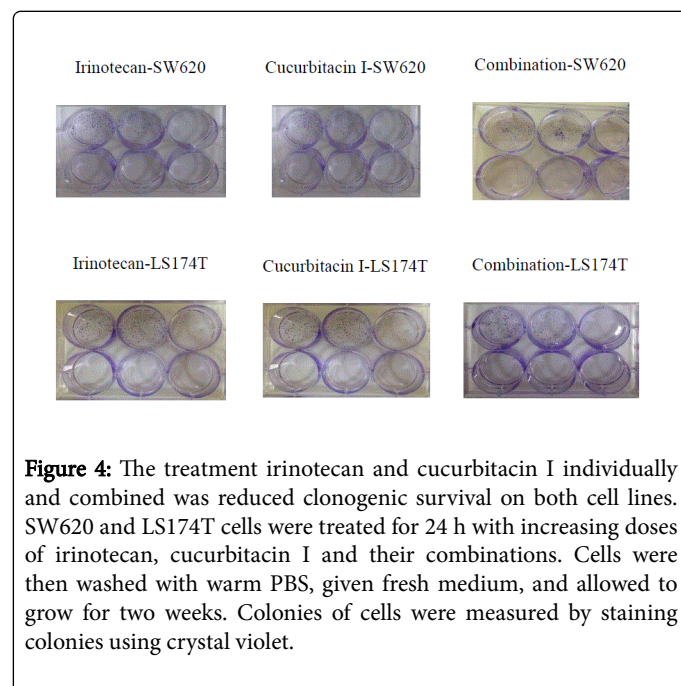

Figure 3: Wound healing responses of colorectal cancer cell lines following exposure to irinotecan, cucurbitacin I and combination. (A) SW620 cells were incubated with irinotecan (1-250 μm). (B) SW620 cells were incubated with cucurbitacin I (1.2-750 nM). (C) SW620 cells were incubated with with irinotecan (1-250 μm) and cucurbitacin I (1.2-750 nM). (D) LS174T cells were incubated with irinotecan (1-250 μm). (E) LS174T cells were incubated with cucurbitacin I (1.2-750 nM). (F) LS174T cells were incubated with with irinotecan (1-250 μm) and cucurbitacin I (1.2-750 nM). The results are reported as the mean ± S.D. Statistical significance is based on the difference when compared with control ($^*P < 0.05$, $^{**}P < 0.01$).

Wound healing test results showed a minimum inhibition of wound healing as compared to the control group at 24 and 48 h in both cancer cell lines and the ability of cell migration was prevented (Figure 4).

Clonogenic assay

The results obtained in the clonogenic assay test supports the MTT test results. We have determined that cucurbitacin I and irinotecan combination therapy destroys the colony forming ability of cancer cells in both cancer cell line.

Figure 4: The treatment irinotecan and cucurbitacin I individually and combined was reduced clonogenic survival on both cell lines. SW620 and LS174T cells were treated for 24 h with increasing doses of irinotecan, cucurbitacin I and their combinations. Cells were then washed with warm PBS, given fresh medium, and allowed to grow for two weeks. Colonies of cells were measured by staining colonies using crystal violet.

Acknowledgment

References

1. Wolpin BM, Mayer RJ (2008) Systemic treatment of colorectal cancer. Gastroenterology 134: 1296-1310.
2. Duzkoylu Y, Bektas H, Sarı YS, Koç O, Bilsel Y, et al. (2013) Comparison of colorectal cancer cases that were diagnosed in emergency operations caused by acute mechanical intestinal obstruction and elective operations. Journal of Diseases of the Colon and Rectum 23: 89-95.
3. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, et al. (2013) Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer 49: 1374-1403.
4. Siegel RL, Miller KD, Jemal A (2015) Cancer Statistics, 2015. CA Cancer J Clin 65: 5-29.
5. Liu T, Zhang M, Zhang H, Sun C, Yang X, et al. (2008) Combined antitumor activity of cucurbitacin B and docetaxel in laryngeal cancer. Eur J Pharmacol 587: 78-84.
6. Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, et al. (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66: 3992-3995.
7. Seal BS, Anderson S, Shermock KM (2016) Factors associated with adherence rates for oral and intravenous anticancer therapy in commercially insured patients with metastatic colon cancer. J Manag Care Spec Pharm 22: 227-235.
8. Bhattacharya D, Easthall C, Willoughby KA, Small M, Watson S (2012) Capecitabine non-adherence: exploration of magnitude, nature and contributing factors. J Oncol Pharm Pract 18: 333-342.
9. Di Bartolomeo M, Ciarlo A, Bertolini A, Barni S, Verusio C, et al. (2015) Capecitabine, oxaliplatin and irinotecan in combination, with bevacizumab (COI-B regimen) as first-line treatment of patients with advanced colorectal cancer. An Italian Trials of Medical Oncology phase II study. Eur J Cancer 51: 473-481.
10. Moreno V, Sanz-Pamplona R (2015) Altered pathways and colorectal cancer prognosis. BMC Med 13: 76.
11. Murray PJ (2007) The JAK-STAT Signaling pathway: Input and output integration. J Immunol 178: 2623-2629.
12. Du W, Hong J, Wang YC, Zhang YJ, Wang P, et al. (2012) Inhibition of JAK2/STAT3 signalling induces colorectal cancer cell apoptosis via mitochondrial pathway. J Cell Mol Med 16: 1878-1888.
13. Alghasham AA (2013) Cucurbitacins-A promising target for cancer therapy. Int J Health Sci 7: 77-89.
14. Song J, Liu H, Li Z, Yang C, Wang C (2015) Cucurbitacin I inhibits cell migration and invasion and enhances chemosensitivity in colon cancer. Oncol Rep 33: 1867-1871.
15. Liu T, Zhang M, Zhang H, Sun C, Deng Y (2008) Inhibitory effects of cucurbitacin B on laryngeal squamous cell carcinoma. Eur Arch Otorhinolaryngol 265: 1225-1232.
16. Chen JC, Chiu MH, Nie RL, Cordell GA, Qiu SX (2005) Cucurbitacins and cucurbitane glycosides: structures and biological activities. Nat Prod Rep 22: 386-399.
17. Dantas IN, Gadelha GC, Chaves DC, Monte FJ, Pessoa C, et al. (2006) Studies on the cytotoxicity of cucurbitacins isolated from Cayaponia racemosa (Cucurbitaceae). Z Naturforsch C 61: 643-646.
18. Recio MC, Prieto M, Bonucelli M, Orsi C, Manez S, et al. (2004) Anti-inflammatory activity of two cucurbitacins isolated from Cayaponia tayuya roots. Planta Med 70: 414-420.
19. Stechishin OD, Luchman HA, Ruan Y, Blough MD, Nguyen SA, et al. (2013) On-target JAK2/STAT3 inhibition slows disease progression in orthotopic xenografts of human glioblastoma brain tumor stem cells. Neuro Oncol 15: 198-207.
20. Lopez-Haber C, Kazanietz MG (2013) Cucurbitacin I inhibits Rac1 activation in breast cancer cells by a reactive oxygen species-mediated mechanism and independently of janus tyrosine kinase 2 and P-Rex. Mol Pharmacol 83: 1141-1154.
21. Hsu HS, Huang PI, Chang YL, Tzao C, Chen YW, et al. (2011) Cucurbitacin I inhibits tumorigenic ability and enhances radiochemosensitivity in nonsmall cell lung cancer-derived CD133-positive cells. Cancer 117: 2970-2985.
22. Chang CJ, Chiang CH, Song WS, Tsai SK, Woung LC, et al. (2012) Inhibition of phosphorylated STAT3 by cucurbitacin I enhances chemoradiosensitivity in medulloblastoma-derived cancer stem cells. Childs Nerv Syst 28: 363-373.
23. Gry J, Soborg I, Andersson HC (2006) Cucurbitacins in plant food. Tema Nord Press.
24. Sun Y, Kang C, Wang M, Zhu J, Jin L, et al. (2016) Nanosized camptothecin conjugates for single and combined drug delivery. Eur J Bio Med Res 2: 8-14.
25. Watters JW, McLeod HL (2003) Cancer pharmacogenomics: current and future applications. Biochim Biophys Acta 1603: 99-111.
26. Park DJ, Won JH, Cho AR, Yun HJ, Heo JH, et al. (2014) Determination of irinotecan and its metabolite SN-38 in rabbit plasma and tumors using a validated method of tandem mass spectrometry coupled with liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 962: 147-152.
27. Liu X, Hummon AB (2015) Quantitative determination of irinotecan and the metabolite SN-38 by nanoflow liquid chromatography-tandem mass spectrometry in different regions of multicellular tumor spheroids. J Am Soc Mass Spectrom 26: 577-586.

28. Lopes F, Smith R, Nash S, Mitchell RT, Spears N (2016) Irinotecan metabolite SN38 results in germ cell loss in the testis but not in the ovary of 2 prepubertal mice. Mol Hum Reprod.

29. Holcombe RF, Kong KM, Wimmer D (2004) Combined topoisomerase I inhibition for the treatment of metastatic colon cancer. Anticancer Drugs 15: 569-574.

30. Fuchs C, Mitchell EP, Hoff PM (2006) Irinotecan in the treatment of colorectal cancer. Cancer Treat Rev 32: 491-503.

31. Liu X, Xu W (2014) UGT1A1*28 polymorphisms: a potential pharmacological biomarker of irinotecan based chemotherapies in colorectal cancer. Pharmacogenomics 15: 1171-1174.

32. Cunningham D, Maroun J, Vanhoefer U, Van Cutsem E (2001) Optimizing the use of irinotecan in colorectal cancer systemic treatment of colorectal cancer. Oncologist 6: 17-23.

33. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, et al. (2000) Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 343: 905-914.

34. Eyol E, Murtaga A, Zhivkova-Galunska M, Georges R, Zepp M, et al. (2012) Few genes are associated with the capability of pancreatic ductal adenocarcinoma cells to grow in the liver of nude rats. Oncol Rep 28: 2177-2187.

35. Liang CC, Park AY, Guan JL (2007) In vitro scratch assay: a convenient and inexpensive method or analysis of cell migration in vitro. Nat Protoc 2: 329-333.

36. Kaleağasıoğlu F, Berger MR (2014) Differential effects of erufosine on proliferation, wound healing and apoptosis in colorectal cancer cell lines. Oncol Rep 31: 1407-1416.

37. Tekedereli I, Alpay SN, Akar U, Yuca E, Ayugo-Rodriguez C, et al. (2013) Therapeutic silencing of Bcl-2 by systemically administered siRNA nanotherapeutics inhibits tumor growth by autophagy and apoptosis and enhances the efficacy of chemotherapy in orthotopic xenograft models of ER (-) and ER (+) breast cancer. Mol Ther Nucleic Acids 2: 121.

38. Lee DH, Iwanski GB, Thoennissen NH (2010) Cucurbitacin: ancient compound shedding new light on cancer treatment. Scientific World J 10: 413-418.

39. Al-Qasem A, Al-Howail HA, Al-Swailem M, Al-Mazrou A, Al-Otaibi B, et al. (2016) PAC exhibits potent anti-colon cancer properties through targeting cyclin D1 and suppressing epithelial-to-mesenchymal transition. Mol Carcinog 55: 233-244.

40. Soto-Hernandez M, Iniguez JC, Arevalo-Galarza LC, Santiago-Osorio E, Aguiniga-Sanchez I, et al. (2015) Lead compounds from cucurbitaceae for the treatment of cancer. Phytochemicals - Isolation, Characterisation and Role in Human Health.

41. Sun Y, Kang C, Zhang A, Liu F, Hu J, et al. (2016) Co-delivery of dual-drugs with nanoparticle to overcome multidrug resistance. Eur J BioMed Res 2: 12-18.

42. Marostica LL, Silva IT, Kratz JM, Persich L, Geller FC, et al. (2015) Synergistic antiproliferative effects of a new cucurbitacin B derivative and chemotherapy drugs on lung cancer cell line A549. Chem Res Toxicol 28: 1949-1960.