Enhancement of 5-Flurouracil Uptake by Insulin in Human Colonic Cancer

Hamid Naji Obeid* Najat A. R. Hasan** Adeeb A. Al-Zubaidy**
*College of Medicine, University of Babylon, Hilla, Iraq.
**College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Received 14 October 2014 **Accepted 13 January 2014**

Abstract

SW-480 cell line was cultivated on two microtiter-plates of 48 wells, by using the routine culture methods and all what needed from culture media and reagents. One microtiter-plate of SW-480 cell line was exposed to 0.5 IU/L of neutral insulin for 10 hours of incubation, followed by serial double dilutions of 5FU starting from 80 µg/mL to 1.25 µg/mL of 5FU for 12 hours incubation. The second cultured plate was exposed to same 5FU serial dilution but without proceeding with insulin. Gas chromatographic analysis was performed to determine the residual 5FU concentration in the culture medium of SW-480 cell line wells in both cultured plates. There was significant differences ($P<0.05$) in the residual 5FU between them. In conclusion: insulin increases the uptake of 5FU into human colonic cancer SW-480 cell line significantly.

الخلاصة

تمت زراعة خط سرطان القولون في طبقين من اطباق المعايرة ذات الثمان وأربعون بئرا بأستخدام الطرق الروتينيه في زراعة الخلايا بكل ماتحتاجه من اوساط زرعيه ومحاليل .عرض أحد طبقي خط خلايا القولون الى نصف وحده دوليه/اللتر من الانسولين المتعادل لفترة حضن قدرها ١٠ ساعات ومن ثم تم تعريضه الى سلسلة من تراكيز مضاعفة التخفيف من عقار الفلوروبوراسيل الخماسي بدءا من ٨٠ مايكروغرام/ مل الى ١,٢٥مايكروكرام/ مل ولمدة ١٢ ساعه حضن. أما الطبق الاخر فقد عرض الى نفس سلسلة تخافيف الفلوروبوراسيل ولكن من دون تعريض مسبق للانسولين. أستخدم جهاز كروماتوغرافيا الغاز في قياس تراكيز الفلوروبوراسيل المتخلفه في الوسط الزرعي لابار خط خلايا القولون في كلا الطبقين المزروعين. لوحظت فروقات معنوية ($P<0.05$) في تراكيز الفلوروبوراسيل المتخلف في كل الطبقين. يستنتج من هذه الدراسة إن الانسولين سبب زياده معنوية في تميثيل عقار الفلوروبوراسيل داخل خلايا خط سرطان القولون البشري.

Introduction

Colorectal cancer (CRC) is a significant health problem; it is the most common malignancy of the gastrointestinal tract [1]. CRC accounts for 10–15% of all cancers [2] , and it is the second leading cause of cancer-related deaths [3]. It is among the top three causes of cancer death in both men and women in the Western world [4]. There are currently eight chemotherapeutic agents licensed for use in treatment of CRC in the US and Europe, one of them is 5FU [5]. It is an antimetabolite of the pyrimidine analogue type [6] which has been frequently used clinically in patients with various cancers [7]. It causes DNA damage, during S phase due to the misincorporation of fluoro-deoxyuridine triphosphate (FdUTP) into DNA[8]. The activity of 5FU is markedly limited by its rapid degradation into $5FUH_2$ via the action of the cytosolic enzyme DPD, the first enzyme in the catabolic cycle of 5FU. It has been demonstrated that this

enzyme deactivates more than 85% of the injected dose of 5FU [9]. The overall response rate for advanced CRC to 5FU alone is still only 10–15% [10].However, nowadays 5FU resistance during the course of treatment has become common, which is an important cause of failure for cancer therapy [11]. To increase the activity of 5FU, various researchers have proposed utilization of biochemical modulators [12] enhancement of 5FU with insulin or insulin potentiation therapy (IPT) was developed by the Mexican medical doctor "Donato Perez Garcia" in the 1930s [13]. IPT utilizes insulin to deliberately induce a hypoglycemic state to starve the cancer cells. However, during the state of hypoglycemia, the starving cancer cells are given low dose chemo agents or natural anti-cancer substances immediately followed by infusion of dextrose sugar. The low dose chemo agents are therefore devoured by and readily absorbed into the cancer cells [14]. Insulin as a metabolic accelerant, may be able to increase the metabolism of cancer cells and stimulate them into a cell cycle stage in which they are more sensitive to anticancer drugs [15] . In addition, insulin promotes the uptake of many nutrition materials by facilitated diffusion like the entry of glucose into many tissues [16].The present study demonstrated the enhancement of intracellular uptake of 5FU by insulin.

Materials and methods

1- Cytotoxicity assays

According to Freshney, (1994) cytotoxicity assays were applied on human colonic cancer SW-480 cell line using MEM culture medium. When the growth in the flask became as monolayer before it reach the exponential phase, the cell monolayer were harvested and re-suspended with a culture medium in a concentration of $5x\ 10^5$ cell / ml and seeded into two microtiter plate. When the cell growth reach 80%, the wells were exposed to 0.5 IU/L of insulin and incubated for 10 h. [17].Then the culture media was changed by fresh one containing 5FU at two-fold serial dilutions (80, 40, 20, 10, 5, 2.5, 1.25 µg/ml). The plates were incubated at 37°C for 12 hours.

2- Gas chromatography analysis

A volume of one ml of cell culture medium was collected from five replicate of each concentration were mixed and centrifuged at 10000 rpm for 10 min [18]. One cubic milliliter of the supernatant was lyophilized and kept overnight in the oven at 50°C for further dryness. The samples were re-suspended with 1mL methanol and analyzed by gas chromatography (Shemadzo 2010-GC) to determine the concentration of 5FU in the culture medium. The injection volume: 1µl, the injector temperature: 280°C, the detector temperature: 300°C and the column oven temperature: 250°C [19]. Results are presented as mean ± standard deviation (SD). A differences was considered to be significant at $P<0.05$ level and is represented by (*).

Results

Figure (1 and 2) shows the standard curve of 5FU detected by Shimadzu GC monitored by florid ion current detector. 5FU standard solutions consisting 100, 50, 25, 12.5, 6.25µg/ml. Figure (3) appears the gas chromatograph of the residual 5FU concentrations in the culture medium of SW-480 cell line wells after treatment with different concentrations of 5FU at 12h incubation. Whereas, Figure (4) appears the gas chromatograph results of the residual 5FU concentrations in the culture medium of SW-480 cell line wells after treatment with different concentrations of 5FU pretreated with 0.5U/L insulin at 12h incubation.

Table (1) appears the residual 5FU concentration in the culture medium of SW-480 cell line wells after 5FU treatment with and without insulin groups. There was significant differences ($P<0.05$) in the residual 5FU between both treated groups.

Discussion

The significant differences in both treated groups indicates that insulin increase the uptake of 5FU into the cells so increase its absorption and consumption. Ke *et al*., (2007) proposed that the detection of 5FU concentration in the culture medium can indirectly analyze the total consumption of 5FU by tumor cells and avoid the effect of drug metabolism and drug efflux via drug pumps at the cell membranes[18] .

Insulin stimulates Na^+, K^+ - ATPase activity, and translocation to the plasma membrane via phosphorylation of the alpha-subunits of insulin receptors by the extracellular signal-regulated kinase [20]. Previous studies demonstrated that the uptake of 5FU is apparently Na^+-dependent. The carrier-mediated transport of 5FU may require some other factors that are yet to be identified [21]. In conclusion, GC analysis confirmed that insulin increased the uptake of 5FU in human colonic cancer SW-480 cell line.

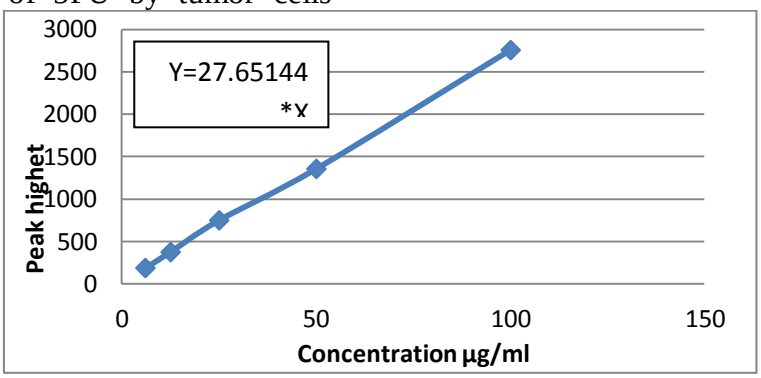


Figure 1 Standard curve for 5-FU stock solutions consisting 100, 50, 25, 12.5, 6.25µg/ml .Shimadzu gas chromatography monitored by florid ion current detection, injector temperature:280 °C, detector temperature :300 °C, Column Oven temperature:250 °C.

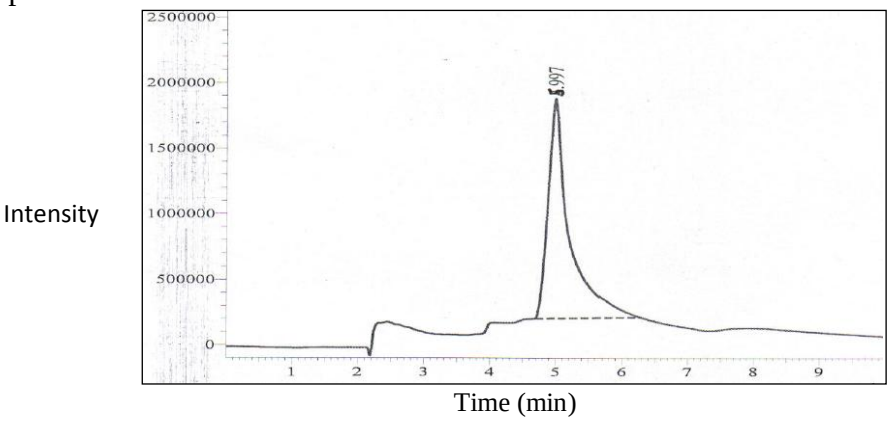
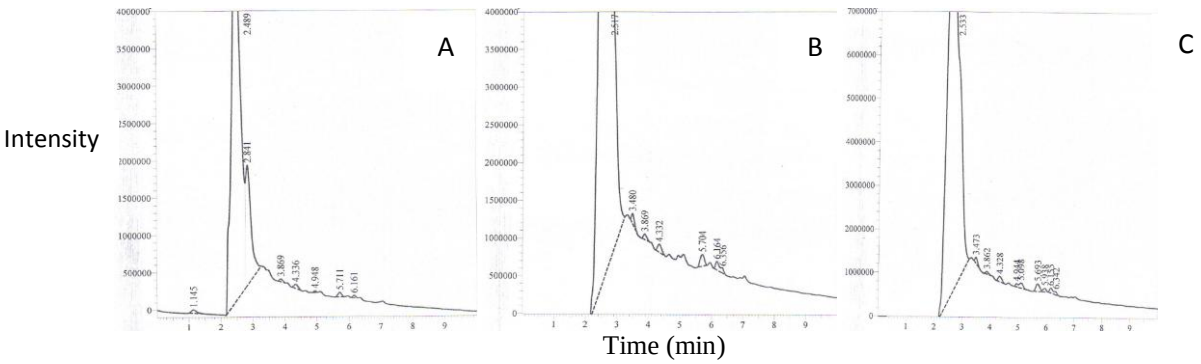
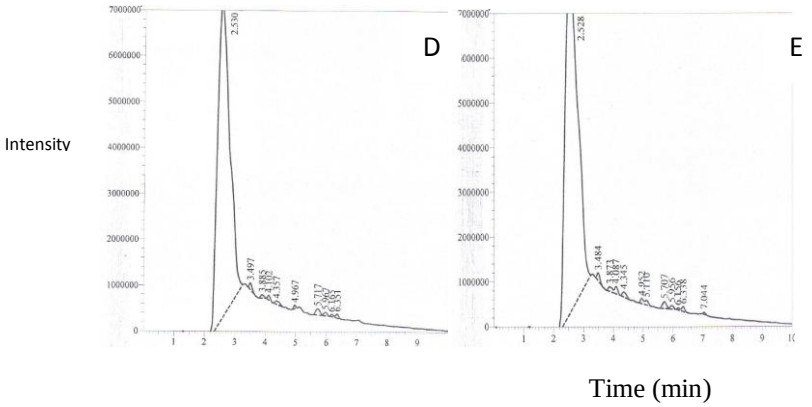


Figure 2 Standard curve for 5-FU. Shimadzu gas chromatography monitored by florid ion current detection, injector temperature:280 °C, detector temperature :300 °C, Column Oven temperature:250 °C.





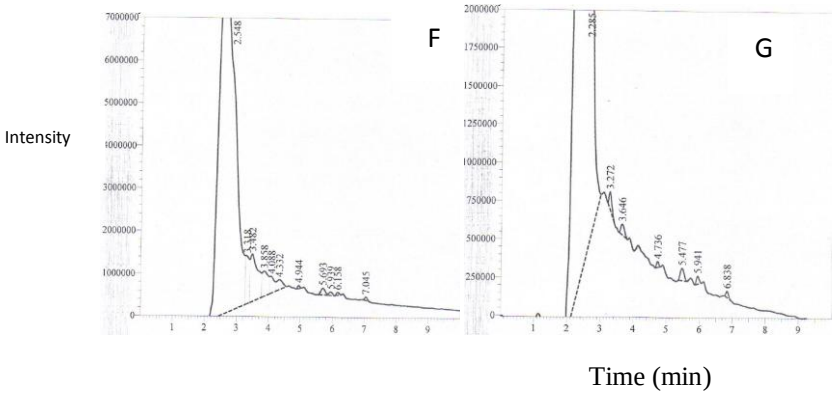


Figure 3 Gas chromatograph of the residual 5FU concentrations in the culture medium of SW-480 cell line wells after treatment with different concentrations of 5FU at 12h incubation.

(A): 80 µg/ml,

(B): 40 µg/ml,

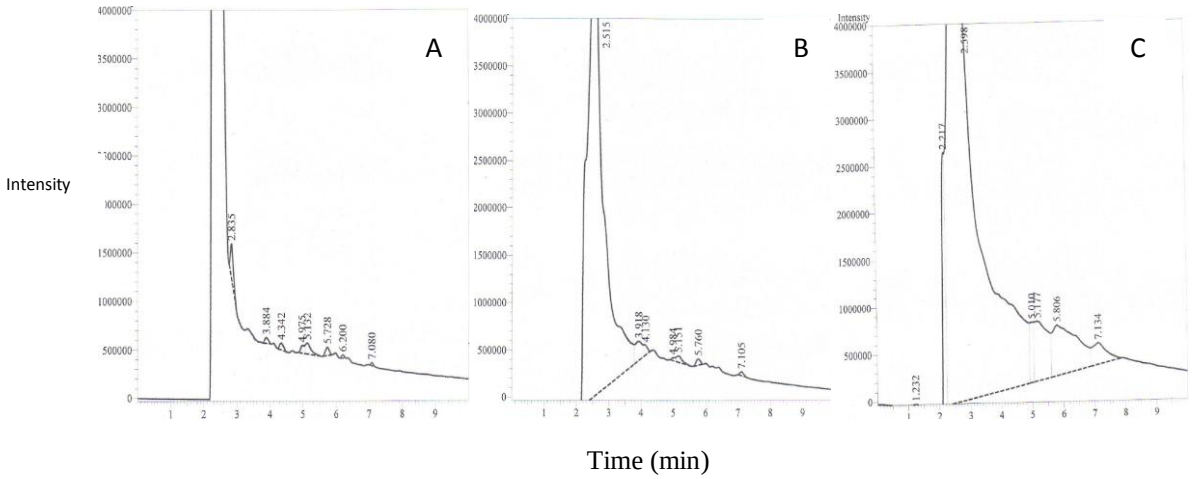
(C): 20 µg/ml,

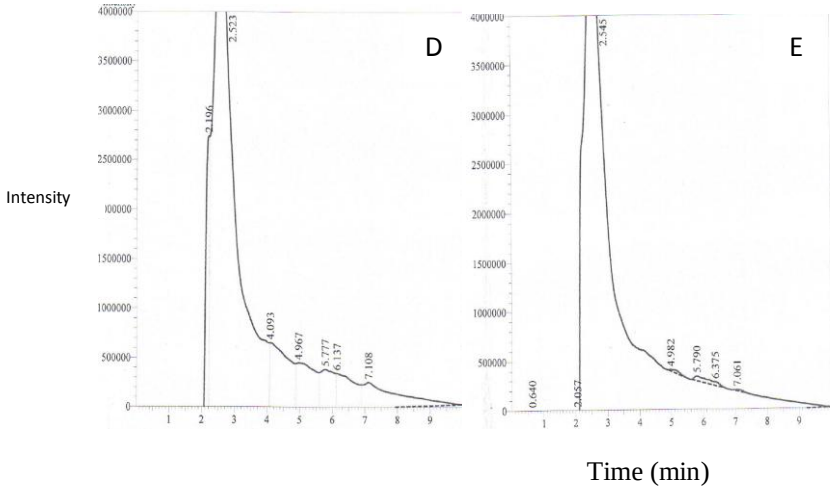
(D): 10 µg/ml,

(E): 5 µg/ml,

(F): 2.5 µg/ml,

(G): 1.25µg/ml





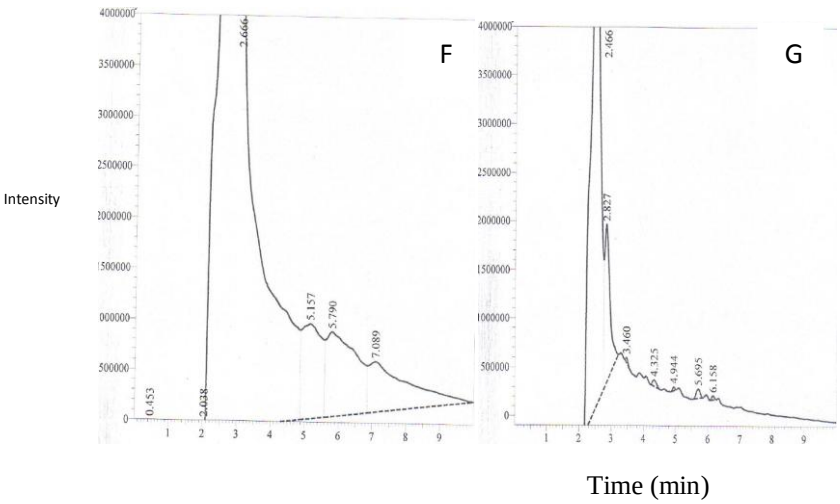


Figure 4 Gas chromatograph of the residual 5FU concentrations in the culture medium of SW-480 cell line wells after treatment with different concentrations of 5FU pre-treated with 0.5U/L insulin for 9h at 12h incubation.

(A): 80 µg/ml,

(B): 40 µg/ml,

(C): 20 µg/ml,

(D): 10 µg/ml,

(E): 5 µg/ml,

(F): 2.5 µg/ml,

(G): 1.25µg/ml.

Table 1 Comparison of residual 5FU concentrations in the culture medium of SW-480 cell line wells after treatment with 5-FU with and without insulin group.

Primary conc. of 5-FU µg/ml	Residual 5-FU conc. (µg/ml) in the medium of SW-480 cell line. Mean ±SD	
	5FU	5FU + insulin (0.5U/L)
1.25	0.060±0.004	0.023±0.009*
2.5	0.122±0.007	0.025±0.011*
5	0.125±0.014	0.059±0.005*
10	0.127±0.011	0.122±0.009*
20	0.137±0.023	0.124±0.012*
40	0.159±0.021	0.143±0.003*
80	0.165±0.015	0.150±0.004*

* = significant difference (P<0.05) between 5FU+insulin and 5FU groups for each concentration.

References

1. Omran, S. and Ismail, AA. (2010). Knowledge and beliefs of Jordanians toward colorectal cancer screening. Cancer Nurs. 33: 141–148.
2. Samantas, E.; Dervenis, C. and Rigatos, S.K.(2007). Adjuvant Chemotherapy for Colon Cancer: Evidence on Improvement in Survival. Dig. Dis.25:67–75.
3. Go, H.; Hwang, H.J. and Nam, T.J. (2010). A glycoprotein from Laminaria japonica induces apoptosis in HT-29 colon cancer cells. Toxicol. *In Vitro*. 24: 1546–1553.
4. Aune, D.; Lau, R.; Chan, DS. et al. (2012). Dairy products and colorectal cancer risk: a systematic review and meta-analysis of cohort studies. Ann. Oncol. 23: 37-45.
5. Davie, S.J.M. and Goldberg, R. M. (2008). First-line therapeutic strategies in metastatic colorectal cancer. Oncology. 22(13):1470-1479.
6. Arias, J. L. (2008). Novel strategies to improve the anticancer action of 5-Fluorouracil by using drug delivery systems. Molecule. 13: 2340-2369.
7. Ide, H.; Kikuchi, E.; Hasegawa, M. et al. (2012). Prognostic significance of 5-fluorouracil metabolism-relating enzymes and enhanced chemosensitivity to 5-fluorouracil by 5-chloro 2,4-dihydroxy-pyridine in urothelial carcinoma. BMC Cancer, 12:420.
8. Peters, GJ.; van Triest, B.; Backus, HH. et al. (2000). Molecular downstream events and induction of thymidylate synthase in mutant and wild-type p53 colon cancer cell lines after treatment with 5-fluorouracil and the thymidylate synthase inhibitor raltitrexed. Eur. J. Cancer . 36:916-924.
9. Guimbaud, R.; Guichard, S. and Dusseau, C. (2000). Dihydropyrimidine dehydrogenase activity in normal, inflammatory and tumour tissues of colon and liver in humans. Cancer Chemother. Pharmacol. 45:477-482.

10. Giacchetti, S.; Perpoint, B.; Zidani, R.et al. (2000). Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracilleucovorin as first-line treatment of metastatic colorectal cancer. J. Clin. Oncol. 18: 136-147.

11. Fukushima, M.; Fujioka, A.; Uchida, J.et al. (2001). Thymidylate synthase and ribonucleotide reductase may be involved in acquired resistance to 5-fluorouracil in human cancer xenografts *in vivo*. Eur. J. Cancer. 37: 1681- 7.

12. Malet-Martino, M. and Martino, R. (2002). Clinical studies of three oral prodrugs of 5-fluorouracil (Capecitabine, UFT, S-1): A Review. The Oncologist.7:288-323.

13. Ayre, S.G.; Perez-Garcia, y.; Bellon, D. and Perez-Garcia, D. (1986). Insulin potentiation therapy: a new concept in the management of chronic degenerative disease. Med. Hypotheses. 20(2):199-210.

14. Simon, YU. (2012). Insulin Therapy for other than Diabetes: Insulin Potentiation Therapy. The Healthy Plante.

15. Wang, R.; Fan, O.; Hu, X. et al. (2008). Insulin-enhanced antitumor effect of 5-fluorouracil *in vivo*. Chin J. Clin. Oncol. 5: 277-280.

16. Gu, S.; Villegas, CJ. and Jiang, JX. (2005). Differential regulation of amino acid transporter SNAT3 by insulin in hepatocytes. J Biol Chem. 280: 26055–62.

17. Freshney, R.I. (1994) Culture of Animal Cells, A Manual of Basic Technique, 3rd ed., New York: Wiley-Liss.

18. Ke, Z.O.U; Ji-hang, J.U. and Hong, X.I. (2007). Pretreatment with insulin enhances anticancer functions of 5-fluorouracil in human esophageal and colonic cancer cells. Acta Pharmacol Sin. 28 (5): 721–730.

19. Kenkel, J. (2000). Analytical Chemistry for Technicians. 3rd edition. Lewis Publishers. A CRC Press Company. Boca Rotan London New York Washington.

20. Al-Khalili, L.; Kotova, O.; Tsuchida, H. et al. (2004). ERK1/2 mediates insulin stimulation of Na(+),K(+)-ATPase by phosphorylation of the alpha-subunit in human skeletal muscle cells. J. Biol. Chem. 279: 25211–8.

21. Yuasa, H.; Matsuhisa, E. and Watanabe, J. (1996). Intestinal brush border transport mechanism of 5-fluorouracil in rats. Biol. Pharm. Bull. 19: 94–9.