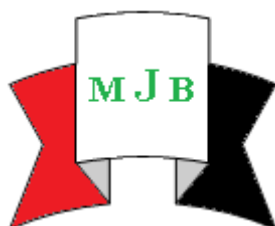


Determination of Glucose Level as a Parameter for Cell Inhibition by 5-Fluorouracil in Human Colonic Cancer SW-480 Cell Line

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Abstract

Colonic cancer (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 serial double dilutions of 5FU starting from 80 µg/mL of 5FU for 12 hours incubation. Optical density was measured after staining with crystal violet and growth inhibition percentage was calculated. Second SW-480 cultured plate was also exposed to same 5-FU dilutions and incubation period, and then the glucose level was determined in the culture medium. There was significant increase ($P < 0.05$) in the inhibition percent and glucose level in SW-480 culture media for 5FU concentrations starting from 2.5µg/ml and up word. In conclusion: According to the result, determination of glucose concentration may be a valuable predictor for the antitumor drug response.

الخلاصة

تمت زراعة خط سرطان القولون الخلوي على طبقي معايرة ذات ٤٨ حفرة باستخدام طرق الزرع الروتينية وما يتطلب من أوساط زرعية ومحاليل. عرض أحد طبقي المعايرة إلى سلسلة تراكيز ثنائية التخفيف من عقار الفلورويوراسيل بدءا من ٨٠ مايكروغرام/مليلتر ولمدة حضن ١٢ ساعة. قيس الكثافة البصرية بعد اجراء عملية التصبيغ بواسطة صبغة الكريستال البنفسجية وبعدها حساب نسبة التثبيط في النمو. عرضت صفيحة ثانيه الى نفس تخافيف الفلورويوراسيل الخماسي ومدة الحضن ومن ثم قيس تراكيز الكلوكوز في الوسط الزرع. لوحظ زيادة معنويه في نسبة التثبيط ومستوى الكلوكوز في وسط خط سرطان القولون لتراكيز الفلورويوراسيل ابتداء من تركيز ٢,٥ مايكروغرام في المليلتر الواحد فما فوق. وطبقا للنتائج فأن قياس تركيز الكلوكوز يعتبر مؤشرا مفيدا لفعالية الأدوية المضادة للسرطان.

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world. An estimated 1.24 million people worldwide were diagnosed with CRC in 2008, accounting for 10% of the total. It is the fourth most common cause of cancer death worldwide, estimated to be responsible for almost 610,000 deaths in 2008 [1]. The incidence of CRC increased around the world during the last two decades even though there were many strategies in

preventing it [2]. 5-Fluorouracil (5FU) is an antimetabolite of the pyrimidine analogue type, with a broad spectrum of activity against solid tumors such as colon cancer [3].

There are various assay methods for evaluating the cytotoxic effects of chemicals on cultured cells including: the neutral red assay [4], methylthiozol tetrazolium (MTT) assay [5] and crystal violet (CV) staining assay [6]. Although used extensively as convenient and rapid measures of cell viability, all of these assay

methods have their disadvantages and must be used with caution [7]. These disadvantages indicate the necessity of using another method for evaluating the cytotoxicity of chemotherapeutic agents. The present study is designed to evaluate glucose level as a parameter for cell viability in comparison with CV assay.

Materials and Methods

1. The effect of 5FU on SW-480

According to Freshny, (1994) *in vitro* method was used to establish the effect of 5FU on human colonic cancer SW-480 cell lines. The frozen cell line was withdrawn and maintained in EME culture medium containing 5% fetal bovine serum (FBS) and antibiotics. When the cell culture forms a monolayer, it was treated with trypsin-EDTA and cell suspension was seeded in a micro titration plates at 5×10^5 cells/ml. Plates then incubated at 37° C until the growth become 80% of confluence. Two-fold serial dilutions of 5FU was prepared (80, 40, 20, 10, 5, 2.5, 1.25 µg/ml). Concisely Five-replicates in the micro titration plate were exposed to 200 µl of each concentrations of 5FU during the log phase of growth and 200 µl of maintenance medium was added to each well of control group. The plates were covered with lids and returned to the incubator at 37° C for 12h [8]. After the end of the exposure period, the medium was decanted off and the culture wells washed gently and twice with phosphate buffer saline (PBS) followed by 10% formalin fixation for twenty minutes. Then the formalin solution washed out and replaced by 100 µg of 0.01% crystal violet dye. After 20 min the remaining

stain was washed gently with tap water for three times. The plate was left until become dry. Then 200 µl of 70% ethanol was added to each well and rocked gently until the solubility CV dye. The optical density of each well was measured by using ELISA reader at 570 nm [9]. The percentage of inhibition was calculated according to the following equation [10] :

Inhibition % = [(optical density of control wells - optical density of test wells) / optical density of control wells] X 100.

2. Determination of glucose level in SW-480 cell line

Glucose concentration was determined in the culture medium of SW-480 cell wells before and after the end of exposure time for treatment with different concentrations of 5FU directly by digital glucose analyzer according to the manufacture procedure.

Results are presented as mean $\pm$ standard deviation (SD). The t-test was used and a differences was considered to be significant at $P < 0.05$ level and is represented by (*).

Results

1-Effects of 5FU on SW-480 cell line

Table (1) shows the inhibition percent of SW-480 cell line for different concentrations of 5FU (0.25, 2.5, 10, 5, 20, 40, 80 µg/ml) after 12h of incubation. It increased significantly ($P < 0.05$) with increasing the concentration of 5FU above 2.5µg/ml as compared with the control group (5FU concentration = 0). Whereas low concentrations of 5FU produce non-significant ($P > 0.05$) increments in inhibition percent.

Table 1 Effect of the treatment with different concentrations of 5FU on the growth of SW-480 cell line after 12h of exposure.

5-FU conc. (µg/ ml)	Inhibition % (mean ± SD)
0 (control)	1.25 ± 0.27
1.25	1.32 ± 0.89
2.5	5.42 ± 2.21
5	8.17 ± 3.44*
10	15.13 ± 5.18*
20	19.98 ± 8.21*
40	30.17 ± 5.56*
80	35.21 ± 8.33*

* = significant differences (P<0.05) as compared with control group.

2- Determination of glucose concentration in culture medium of SW-480 cell line

Table (2) shows that the concentration of glucose in SW-480 cell line treated with different concentrations of 5FU (1.25, 2.5, 5, 10,

20, 40, 80µg/ml) were (49 ± 5.81, 50 ± 4.11, 52 ± 3.31, 54 ± 2.33, 57 ± 4.14, 70 ± 3.33, 73 ± 2.45, 149 ± 5.33) respectively. There was significant increase (P<0.05) in glucose level for concentrations above 2.5µ/ml as compared with the control group.

Table 2 Glucose concentration in the culture medium of SW-480 cell line after treatment with different concentrations of 5FU.

5-FU conc. (µg/ml)	Determined glucose conc.(mg/dl) (mean ± SE)
0	49 ±5.81
1.25	50 ± 4.11
2.5	52 ± 3.31
5.0	55 ±2.33*
10	57 ± 4.14*
20	70 ±3.33*
40	73 ±2.45*
80	149 ±5.33*

*= significant differences (P<0.05) as compared with the control group.

Discussion

Table (1) shows that the low concentrations of 5FU (1.25 and 2.5µg/ml) produced non-significant (P>0.05) increase in the inhibition percent of SW-480 cell line after 12h of incubation. Whereas concentrations

above 2.5µg/ml produced significant (P<0.05) increase in the inhibition percent of the cells. This indicated that the effect of 5FU on SW-480 was dose dependent. This result was in agreement with Schuler *et al.*, (2010) who obtained that 5FU inhibited the

growth of head and neck squamous cell cancer in a dose dependent manner [11]. Midena *et al.*, (2013) also found that the cytostatic effect of 5FU on human corneal epithelial cell and human corneal keratocyte cultures was time and dose dependent [12].

There was significant increase ($P<0.05$) in the glucose level for 5FU concentrations above 2.5 μ l/ml as compared with the control group (Table 2). This may ensure the growth inhibition of SW-480 cell line by these concentration of 5FU, because cancer cells require a steady source of metabolic energy in order to continue their uncontrolled growth and proliferation [13].

The comparison of this result with the result in table (1) revealed that there is relationship between the effect of 5FU concentrations and the glucose level on SW-480 cell line. Su *et al.*, (2013) investigated whether tumors responding to epidermal growth factor receptor kinase inhibitors can be identified by measuring treatment-induced changes in glucose utilization. They concluded that glucose metabolic activity closely reflects the response to gefitinib therapy [14]. Glucose determination may be a valuable predictor antitumor response.

References

1. Ferlay, J.; Shin, HR.; Bray, F. *et al.* (2010). Colorectal cancer incidence, mortality and prevalence worldwide in 2008 - summary. International Agency for Research on Cancer.
2. Giovannucci, E. and Michaud D. (2007). The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology*.132:2208–2225.
3. Arias, J. L. (2008). Novel strategies to improve the anticancer action of 5-Fluorouracil by using drug delivery systems. *Molecule*. 13: 2340-2369.
4. Borenfreund, E. and Puerner, J. A. (1985). Toxicity determined in vitro by morphological alterations and neutral red absorption. *Toxicology Letters*. 24: 119-124.
5. Denizot, F. and Lang, R. (1986) Rapid colorimetric assay for cell growth and survival: Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods* 89, 271-277.
6. Itagaki, H.; Hagino, S. Kobayashi, T. *et al.* (1991). An *in vitro* alternative to the Darize eye irritation test: evaluation of the crystal violet staining method. *Toxicology in Vitro*. 5: 139-143.
7. Chiba, K.; Kawakami, K. and Tohyama, K. (1998). Simultaneous Evaluation of Cell Viability by Neutral Red, MTT and Crystal Violet Staining Assays of the Same Cells. *Toxicology in vitro*. 12: 251-258.
8. Freshney, R.I. (1994) *Culture of Animal Cells, A Manual of Basic Technique*, 3rd ed., New York: Wiley-Liss.
9. Castro-Garza, J.; Barrios-García, H.B.; Cruz-Vega, D. *et al.* (2007). Use of a colorimetric assay to measure differences in cytotoxicity of *Mycobacterium tuberculosis* strains. *Journal of Medical Microbiology*. 56, 733–737.
10. Chaing, W.; Chang, MY. And Lin, CC. (2003). *In vitro* cytotoxic antiviral and immunomodulatory effects of *Plantago major* and *Plantago asiatica*. *American Journal of Chinese Medicine*. 31(2): 225- 234.
11. Schuler, PJ.; Trellakis, S.; Greve, J. *et al.* (2010). *In vitro* chemosensitivity of head and neck cancer cell lines. *Eur. J. Med. Res*. 15(8): 337–344.

12. Midena, E.; Lazzarini, D.; Catania, AG. *et al.* (2013). Cytostatic and cytotoxic effects of 5-fluorouracil on human corneal epithelial cells and keratocytes. 32(3):338-44.
13. Airley, R E. and Mobasheri, A. (2007). Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. *Chemotherapy*. 53(4): 233-256.
14. Su, H.; Bodenstein, C.; Dumont, R. A. *et al.* (2013). Monitoring Tumor Glucose Utilization by Positron Emission Tomography for the Prediction of Treatment Response to Epidermal Growth Factor Receptor Kinase Inhibitors. *Clinical Cancer Research*. 19 (16): 4293.