

Original article:**ROSIGLITAZONE DIMINISHES THE HIGH-GLUCOSE-INDUCED MODULATION OF 5-FLUOROURACIL CYTOTOXICITY IN COLORECTAL CANCER CELLS**

Meng-Fei Lau^{1,3}, Shalini Vellasamy¹, Kek-Heng Chua^{1,3}, Vikineswary Sabaratnam^{2,3}, Umah Rani Kuppusamy^{1,3*}

¹ Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

² Institute of Biological Science, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

³ Mushroom Research Centre, University of Malaya, 50603 Kuala Lumpur, Malaysia

* Corresponding author: Prof. Dr. Umah Rani Kuppusamy, Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. Tel: +603 7967 6616/+603 7967 4900. Fax: +603 7967 6600. E-mail: umah@um.edu.my

<http://dx.doi.org/10.17179/excli2018-1011>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

ABSTRACT

Colorectal cancer (CRC) is the third most leading cause of morbidity and mortality throughout the world. 5-fluorouracil (5-FU), which is often administrated to disrupt carcinogenesis, was found to elevate blood glucose level among CRC patients. Thus, this study was conducted to evaluate the influence of rosiglitazone on antiproliferative effect of 5-FU using cellular model. Two human colonic carcinoma cell lines (HCT 116 and HT 29) were cultured in the presence of 5-FU, rosiglitazone or in combination under normal and high glucose concentration. The drug cytotoxicity was evaluated using the MTT assay whereas the assessment of cell cycle was carried out using the flow cytometry technique. Combination index (CI) method was used to determine the drug interaction between rosiglitazone and 5-FU. High glucose diminished the cytotoxic effect of 5-FU but at a high drug dosage, this effect could be overcome. Cell cycle analysis demonstrated that 5-FU and rosiglitazone caused G1-phase arrest and S-phase arrest, respectively. CI values indicated that rosiglitazone exerted synergistic effect on 5-FU regardless of glucose levels. This study is the first to demonstrate the influence of rosiglitazone on cytotoxicity of 5-FU under normal or high glucose level. Rosiglitazone may be a promising drug for enhancing the efficacy of 5-FU in the treatment of CRC associated with hyperglycemia.

Keywords: rosiglitazone, 5-fluorouracil, high glucose, MTT, cell cycle, colorectal cancer cell

INTRODUCTION

Colorectal cancer (CRC) is the third most leading cause of morbidity and mortality throughout the world (Ferlay et al., 2015). It is estimated that over one million new cases are diagnosed per year, which accounts for around 10 % of all cancer incidences in both sexes.

Chemotherapy with curative intent is often applied as a first-line treatment to disrupt carcinogenesis. In this context, 5-fluorouracil (5-FU) plus leucovorin is the main option for CRC patients (Goodwin and Asmis, 2009). Other active agents such as irinotecan, capecitabine, oxaliplatin, bevacizumab, cetuximab or panitumumab are also adopted in

fluorouracil-based therapy to improve overall survival and reduce the risk of disease recurrence. Unfortunately, the clinical efficacy is highly dose-dependent due to pharmacokinetic variability and it causes severe toxicities in some CRC patients despite the administration of standard drug protocol (André et al., 2004).

Based on previous epidemiological studies, diabetes mellitus is suggested to be an independent risk factor for colorectal cancer. Although the relationship is not entirely understood, most findings concur with a positive association between diabetes and colorectal cancer (de Bruijn et al., 2013; Deng et al., 2012; Krämer et al., 2012; Larsson et al., 2005; Luo et al., 2012; Mills et al., 2013). Giovannucci et al. (2010) reported that plausible biological mechanisms underlying this association can be attributed to the effect of hyperglycemia, hyperinsulinemia or inflammation on cancer aetiology and progression. An improved glucose control should be an important therapeutic approach for CRC patients.

Besides metformin, thiazolidinediones including rosiglitazone and pioglitazone are another class of oral antidiabetic drugs which help to protect against hyperglycemia (Yki-Järvinen, 2004). Thiazolidinediones increase insulin sensitivity by activating one or more peroxisome proliferator-activated receptors (PPARs) to regulate glucose utilization and production. Several *in vitro* studies indicated that rosiglitazone acts as a PPAR-gamma agonist which suppresses cell proliferation, inhibits cell invasiveness, arrests cell cycle and induces apoptosis in cancer cell lines (Cao et al., 2009, 2015; Han and Roman, 2006; He et al., 2008; Lin et al., 2007; Zhang et al., 2008a). These anticancer properties provoke the use of rosiglitazone on individuals who developed diabetic complications during or after fluorouracil-based regimen.

A recent meta-analysis showed a decreased risk of colorectal cancer incidence when rosiglitazone was administered to diabetic patients (Monami et al., 2014). While high glucose can modulate cytotoxicity of 5-

FU (Ma et al., 2014), the efficacy of rosiglitazone is possibly affected by the level of hyperglycemic condition. In this study, the influence of rosiglitazone on 5-FU pretreated human colon cancer cell lines was evaluated at high glucose level. The drug interaction between rosiglitazone and 5-FU was also determined.

MATERIAL AND METHODS

Reagents

Blank glucose Dulbecco's Modified Eagle Medium (DMEM) (Cat. no.: 11966) and normal glucose DMEM (Cat. no: 11885) were purchased from ThermoFisher. High glucose DMEM (D5671 Sigma) was complemented with 4.0 mM L-glutamine, 1.0 mM sodium pyruvate, 10 % fetal bovine serum, 1 % penicillin streptomycin and 1 % amphotericin B. The stock solutions of 5-FU (F6627 Sigma) and rosiglitazone (R2408 Sigma) were prepared in phosphate-buffered saline (PBS) and dimethyl sulfoxide (DMSO) with a concentration of 5 mg/ml and 1 mg/ml respectively. MTT formazan powder (M5655 Sigma) was dissolved in PBS and diluted to 5 mg/ml. Propidium iodide (PI, Cat. no: P1304MP) and RNase A (12091-021) were obtained from ThermoFisher.

Cell culture

Two human colonic carcinoma cell lines, namely HCT 116 and HT 29, were cultured in normal glucose DMEM (Cat No: 11885) supplemented with 10 % fetal bovine serum, 1 % penicillin streptomycin and 1% amphotericin B. A human normal colon cell line (CCD-18Co) was cultured in normal glucose Minimum Essential Medium Eagle (MEME, D2279 Sigma) complemented with 4.0 mM L-glutamine, 1.0 mM sodium pyruvate, 20 % fetal bovine serum, 1 % non-essential amino acid and 1 % penicillin streptomycin. All cell lines were maintained in humidified atmosphere of 5 % CO₂ at 37±2 °C.

Cell proliferation assay

HCT 116 cells, HT 29 cells and CCD-18Co cells were seeded into 96-well plates at a density of 2000 cells/100 μ l, 2500 cells/100 μ l and 3500 cells/100 μ l per well respectively. After incubation at 37 \pm 2 $^{\circ}$ C for 24 h, the cells were then treated with 5-FU or rosiglitazone at various concentrations for 48 h. Next, 10 μ l of MTT was added and the plates were incubated in the dark at 37 \pm 2 $^{\circ}$ C for 3 h. After solubilizing the formazan crystals in 100 μ l DMSO, absorbance was measured at 560 nm. The cell proliferation was calculated as follows: {[absorbance of treated group-absorbance of blank] / [absorbance of control group-absorbance of blank]} \times 100. The IC₂₀ and IC₅₀ were determined through linear regression analysis.

Cell cycle assay

For the cell cycle assay, drug treatment was carried out in 12-well culture plates with a density of 40,000 cells/ml/well (HCT 116) and 55,000 cells/ml/well (HT 29) at 37 \pm 2 $^{\circ}$ C for 48 h. The cells were harvested and fixed in 2 ml of cold 70 % (v/v) absolute ethanol and stored overnight at -20 $^{\circ}$ C. Then, the fixed cells were washed twice with PBS. Cell staining was done by sequentially adding 450 μ l PBS, 25 μ l RNase A (1 mg/ml) and 50 μ l PI (0.1 mg/ml). The stained cells were incubated in the dark at room temperature (25 \pm 2 $^{\circ}$ C) for 30 min and further examined by a flow cytometer (BD FACScanto II) integrated with BD FACSDiva Software. Modfit LT 2.0 was used to analyze DNA content histograms.

Experimental design

First, the cell proliferation of HCT 116 and HT 29 under normal glucose (NG, 5.5mM) and high glucose (HG, 25mM) culture conditions for 48 h was carried out. The complete blank glucose DMEM containing 1.0 mM sodium pyruvate, 10 % fetal bovine serum, 1 % penicillin streptomycin and 1 % amphoterin B served as a negative control whereas NG DMEM+19.5 mM D-mannitol and blank glucose DMEM+25 mM D-

mannitol were the osmotic controls. The cell proliferation of CCD-18Co cells was evaluated at different glucose concentrations with NG MEME+19.5 mM D-mannitol as an osmotic control.

To study the effect of glucose on drug cytotoxicity, both cancer and normal cells lines were pre-treated with 0.2 μ g/ml 5-FU for 24 h. Then, spent culture media was aspirated and replaced with the following experimental media: (i)NG medium+0.2 μ g/ml 5-FU; (ii)NG medium+2 μ g/ml 5-FU; (iii)HG medium+0.2 μ g/ml 5FU; (iv)HG medium+2 μ g/ml 5-FU. The control group was 5-FU pre-treated cells in NG medium without any drug treatment for subsequent 48 h incubation. The same experiment was repeated with treatments of rosiglitazone alone and a combination of 5-FU and rosiglitazone. In this study, low dose was defined as a concentration close to IC₂₀ of the drug (Kashif et al., 2015).

Statistical analysis

SPSS Statistics 17.0 software, ANOVA followed by Duncan Multiple Range Test was performed to analyse the data. Value of $p < 0.05$ was considered statistically significant. All results were expressed as mean \pm SEM from three independent experiments of at least three replicates.

RESULTS

Effect of 5-FU and rosiglitazone on cell proliferation

Figure 1 shows the cell proliferation of HCT 116 and HT 29 cells upon treatment with increasing concentrations of 5-FU or rosiglitazone. Both cell lines showed a dose-dependent decrease in cell proliferation. HCT 116 responded to 5-FU (Figure 1A₁) at IC₅₀= 4.77 \pm 0.55 μ g/ml and rosiglitazone (Figure 1B₁) at IC₅₀= 11.61 \pm 3.49 μ g/ml. The respective drugs resulted in lower IC₅₀ values of 1.86 \pm 0.18 μ g/ml (5-FU, Figure 1A₂) and 1.04 \pm 0.14 μ g/ml (rosiglitazone, Figure 1B₂) on HT 29. Since the IC₂₀ of both drugs was comparable between HCT 116 and HT 29, a low dose of 5-FU at 0.2 μ g/ml and rosiglita-

zone at 0.5 µg/ml were selected. 5-FU and rosiglitazone did not exert any significant effect at the low dose range but showed 30-40 % inhibition at extremely high concentration (>10 µg/ml) on CCD-18Co cells (Figures 1A₃ and 1B₃).

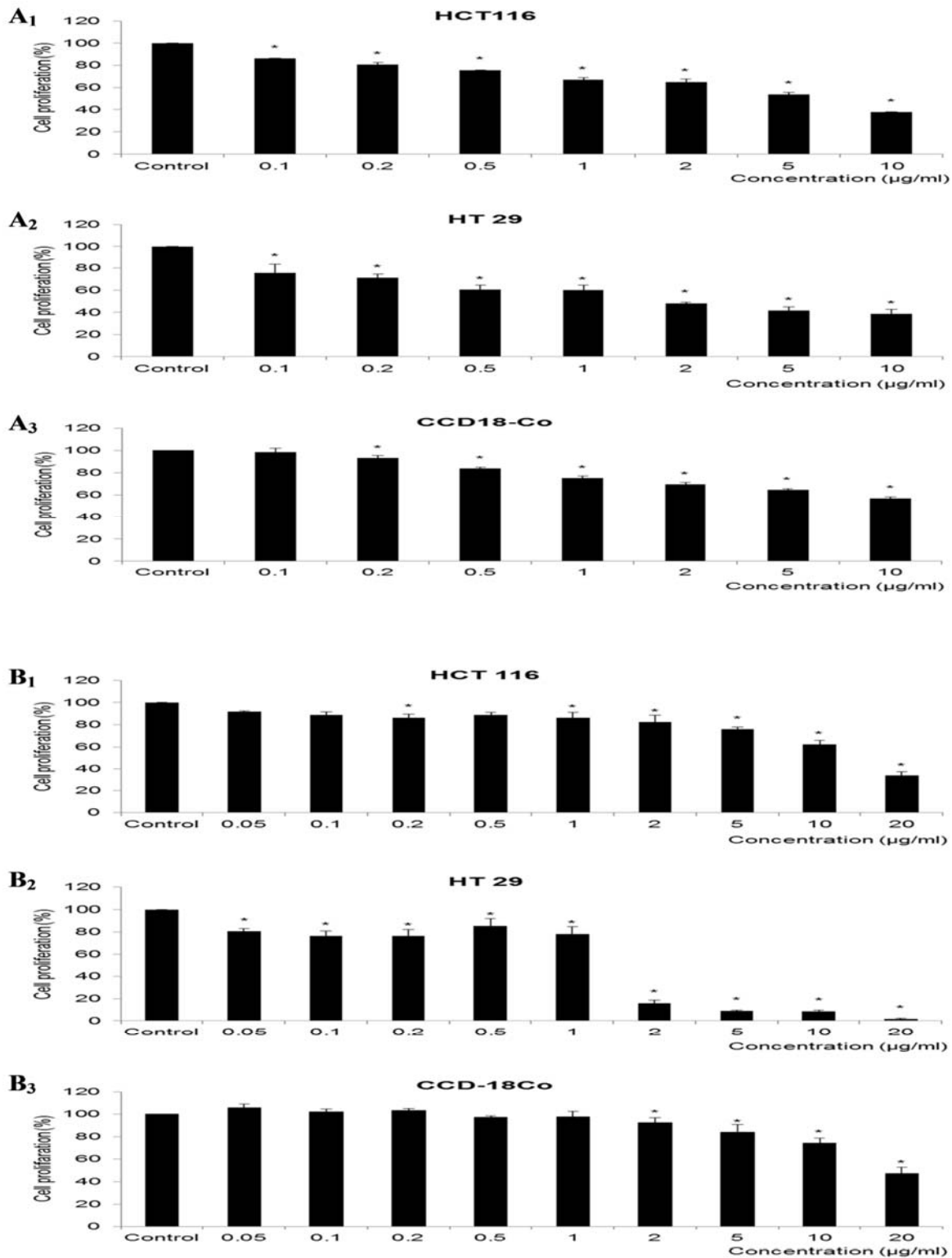


Figure 1: Effect of drug treatment on the cell proliferations. Cells were treated with (A) 5-FU or (B) rosiglitazone at various concentrations for 48 h. The control group were untreated cells in normal glucose medium. *Compared to control, $p < 0.05$.

In HG culture, the cell proliferation was stimulated significantly 0.7 fold (HCT116, Figure 2A₁) and 1.6 fold (HT29, Figure 2A₂)

compared with NG culture. Subsequent introduction of high glucose (25 mM) even reduced the inhibitory effect of 5-FU.

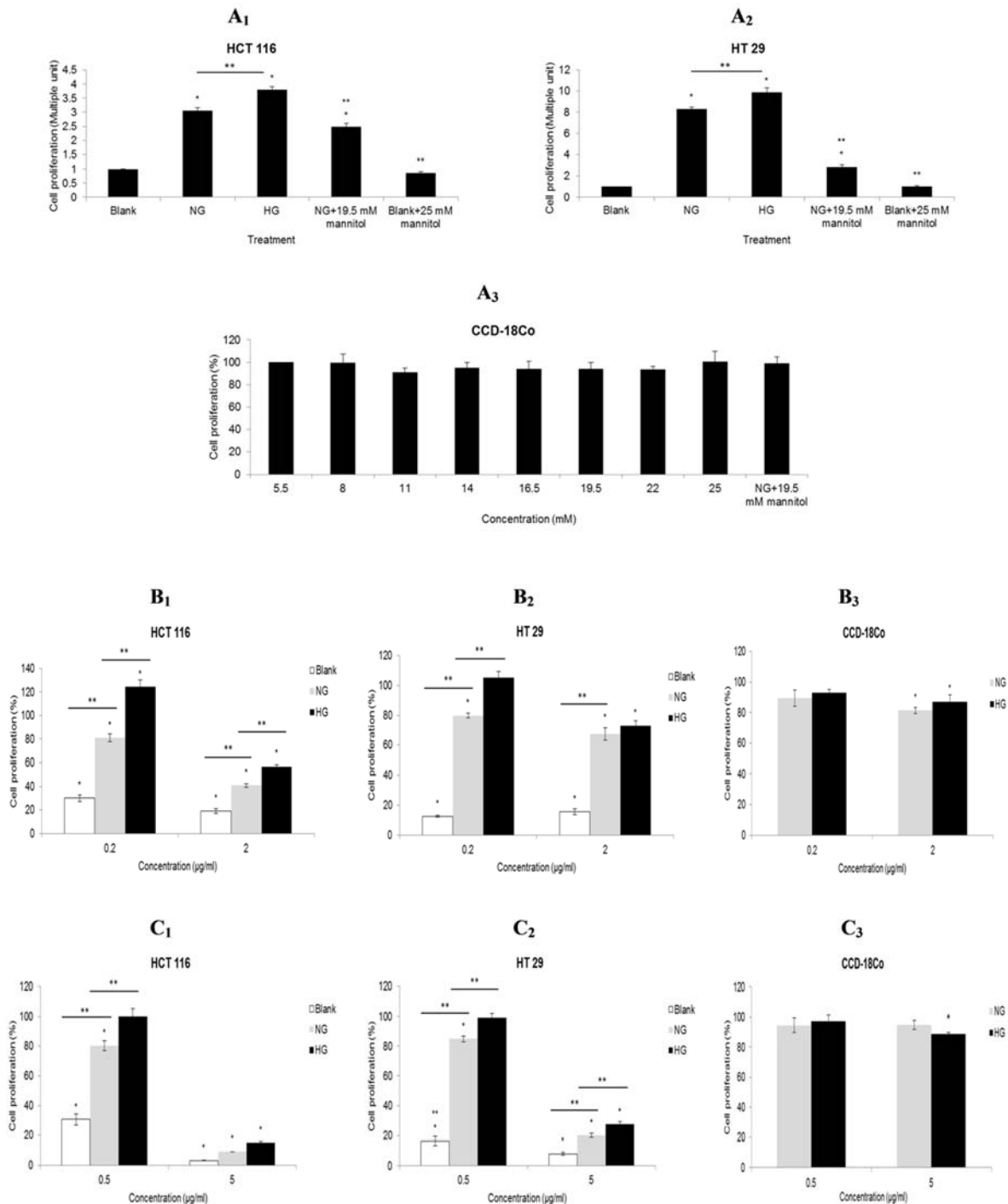


Figure 2: Effect of glucose on drug treatments. (A) HCT 116 and HT29 cells were treated in NG or HG culture for 48 h. The control group were cells incubated in blank medium. Two media containing mannitol with a concentration of iso-osmolar to HG was used as osmotic controls. CCD-18Co cells were cultured at different glucose concentrations. (B-C) 5-FU pretreated cells with subsequent low dose or high dose of drug treatments in blank (absent on CCD-18Co), NG and HG media for 48 h. The control group were 5-FU pre-treated cells in NG culture without any subsequent drug treatments. *Compared to control, $p < 0.05$. **Compared to NG group, $p < 0.05$. NG, normal glucose (5.5 mM); HG, high glucose (25 mM) Panel B: 5-FU, low dose =0.2 µg/ml; high dose =2 µg/ml, Panel C: Rosiglitazone, low dose =0.5 µg/ml; high dose =5 µg/ml

At low dose of 5-FU (0.2 µg/ml), HG culture significantly ($p < 0.05$) increased the cell proliferation of HCT 116 up to 124 % (Figure 2B₁). The cell proliferation of HT 29 was approximately 105 % in HG culture and it was significantly ($p < 0.05$) higher than that in NG culture (80 %) (Figure 2B₂). At low dose of rosiglitazone (0.5 µg/ml), a significant difference ($p < 0.05$) in cell proliferations between HG culture (HCT 116= 100%; HT 29= 99 %) and NG culture (HCT 116= 80 %; HT 29= 85 %) was evident (Figures 2C₁ and 2C₂). The viability of both cell lines was less than 30 %, either in HG or NG culture, when treated with a high dose of rosiglitazone (5 µg/ml). Glucose level had no significant effect on CCD-18Co cells regardless of drug treatment (Figures 2B₃ and 2C₃).

Figures 3A and 3B show the cell proliferations of HCT 116 and HT 29 respectively when 5-FU and rosiglitazone were used in combination. At low dose of 5-FU, an addition of low dose rosiglitazone significantly ($p < 0.05$) decreased the cell proliferation of HCT 116 from 118 % to 96 % in HG culture while the same treatment had no effect on HT 29. At high dose of 5-FU, an addition of low dose rosiglitazone in HG culture triggered the cells to proliferate at an equivalent rate to those treated with single dose of 5-FU. The two cell lines maintained viability below 20 % with combined drugs containing high dose of rosiglitazone in NG and HG cultures. Similarly, glucose level had no significant effect on CCD-18Co cells regardless of drug treatment (Figure 3C).

Effect of 5-FU and rosiglitazone on cell cycle

Exposure to low dose 5-FU caused G1 phase arrest on HCT 116 cells cultured in NG (82.02±1.35 %) and HG (62.83±0.99 %) conditions (Figure 4). However, low dose rosiglitazone arrested the cells in S phase in HG culture (52.25±1.70 %). In the presence of both 5-FU and rosiglitazone, G1 phase

arrest was observed in NG (78.06±0.74 %) with S-phase cells significantly ($p < 0.05$) increased from 12.27±1.57 % to 21.26±0.73 % and HG (61.01±1.17 %) cultures. Under the similar treatments, HG culture significantly increased the cell proportion in S phase when compared to those in NG culture.

In NG culture, HT 29 cells treated with low dose 5-FU were arrested in S phase (50.62±0.94 %) as shown in Figure 5. Low dose of rosiglitazone induced G1 phase arrest but significantly ($p < 0.05$) increased the proportion of S phase-cells from 30.37±1.66 % to 37.97±1.84 %. Treatment with 5-FU or rosiglitazone had no effect on the cell cycle in HG culture. The significant ($p < 0.05$) increase in cell proportion, 43.16±1.52 % (NG) and 30.32±2.46 % (HG), was observed in S phase when treated with both drugs together. Regardless of drug treatment, HG culture significantly ($p < 0.05$) increased the cell proportion in G2 phase when compared with NG culture.

DISCUSSION

Warburg hypothesis postulates that the origin of carcinogenesis is a change of metabolism which is often associated with enhanced glucose uptake and consumption in cancer cells (vander Heiden et al., 2009). In this respect, hyperglycemia can be a primary glucose source to meet the metabolic demand of fast growing cancerous cells. Several *in vitro* studies have demonstrated that high glucose levels promote the proliferation of human colorectal carcinomas (Ma et al., 2014; Masur et al., 2011; Tomas et al., 2012) which concur with the result obtained in this study. The increase in cell proliferation could be independent of osmotic stress as it was reproduced by D-mannitol with a significant difference against normal glucose. It was differed to normal cells which had no respond to glucose level (Figure 2A₃).

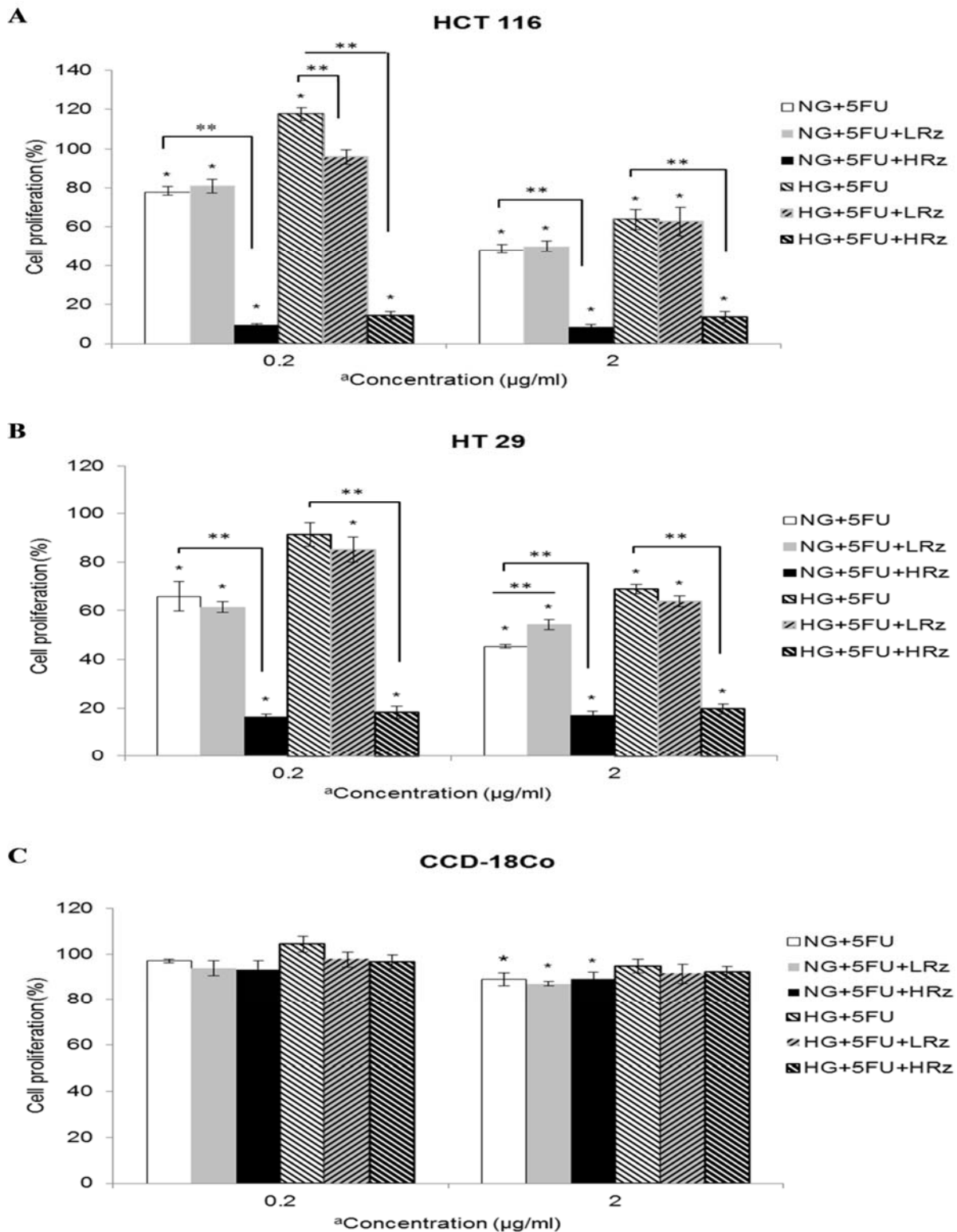


Figure 3: Effect of combined drugs on cell proliferation under NG or HG culture. 5-FU pretreated cells were further exposed to 5-FU alone or in combination with rosiglitazone for 48 h incubation. The control group were 5-FU pre-treated cells in NG culture without any subsequent drug treatments.

*Compared to control, $p < 0.05$. **Compared to single dose 5-FU, $p < 0.05$.

NG, normal glucose (5.5 mM); HG, high glucose (25 mM)

^a0.2 µg/ml as low dose 5-FU; 2 µg/ml as high dose 5-FU

LRz, low dose rosiglitazone= 0.5 µg/ml

HRz, high dose rosiglitazone= 5 µg/ml

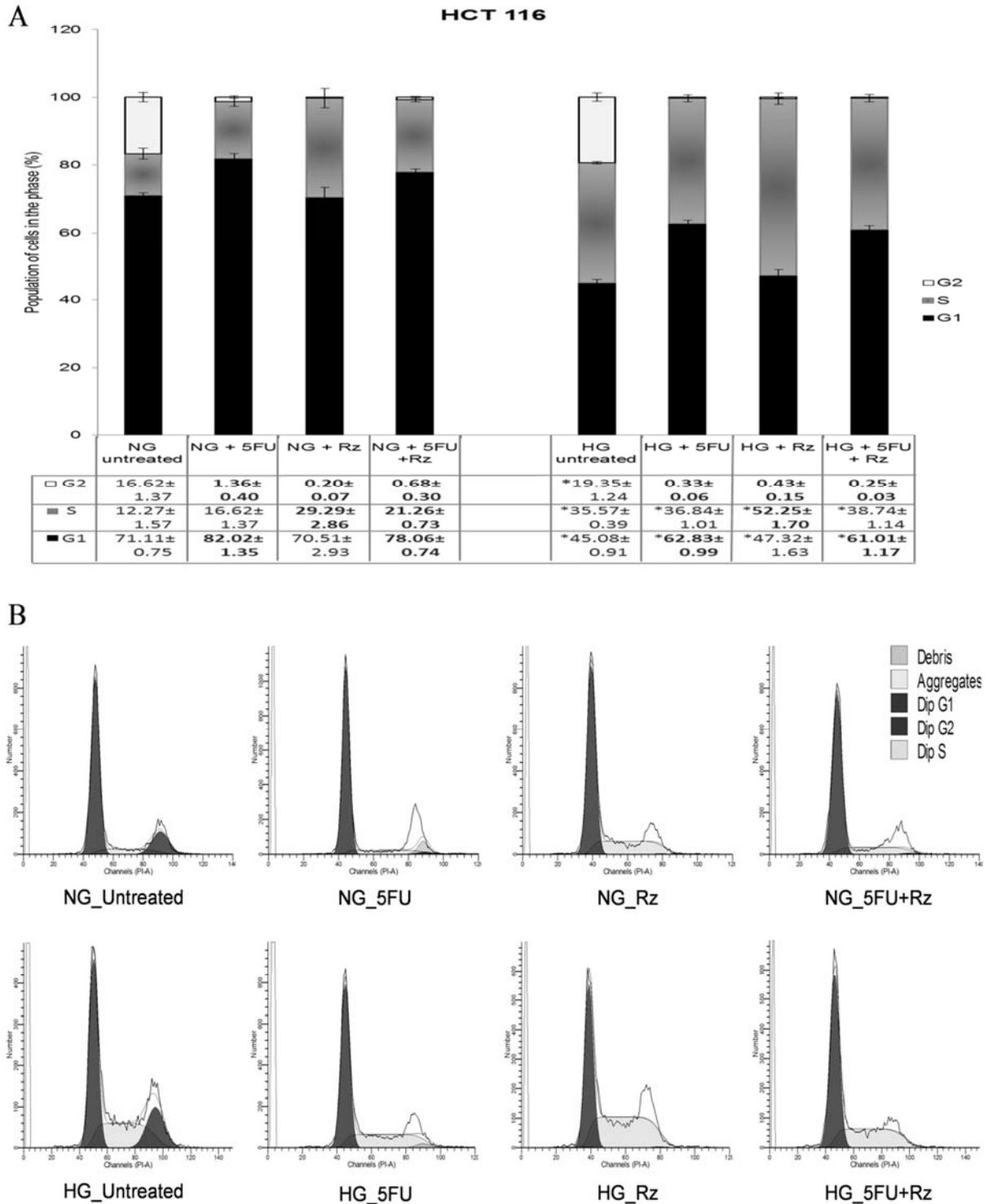


Figure 4: 5-FU pretreated cells (HCT 116) were exposed to different drug treatments for 48 h incubation in the present of normal glucose (NG) or high glucose (HG). **(A)** Data are presented as mean±SEM from three independent experiments in at least three replicates. Numbers in bold are significantly different compared to the values of untreated cells, $p < 0.05$. **(B)** Representative DNA content histograms analyzed by Modfit software.

*Compared to NG group with the same treatment, $p < 0.05$.

NG, normal glucose (5.5 mM); HG, high glucose (25 mM)

Concentration of 5-FU= 0.2 µg/ml; Concentration of rosiglitazone= 0.5 µg/ml

G1: Interphase Gap1; S: Interphase Synthesis; G2: Interphase Gap2

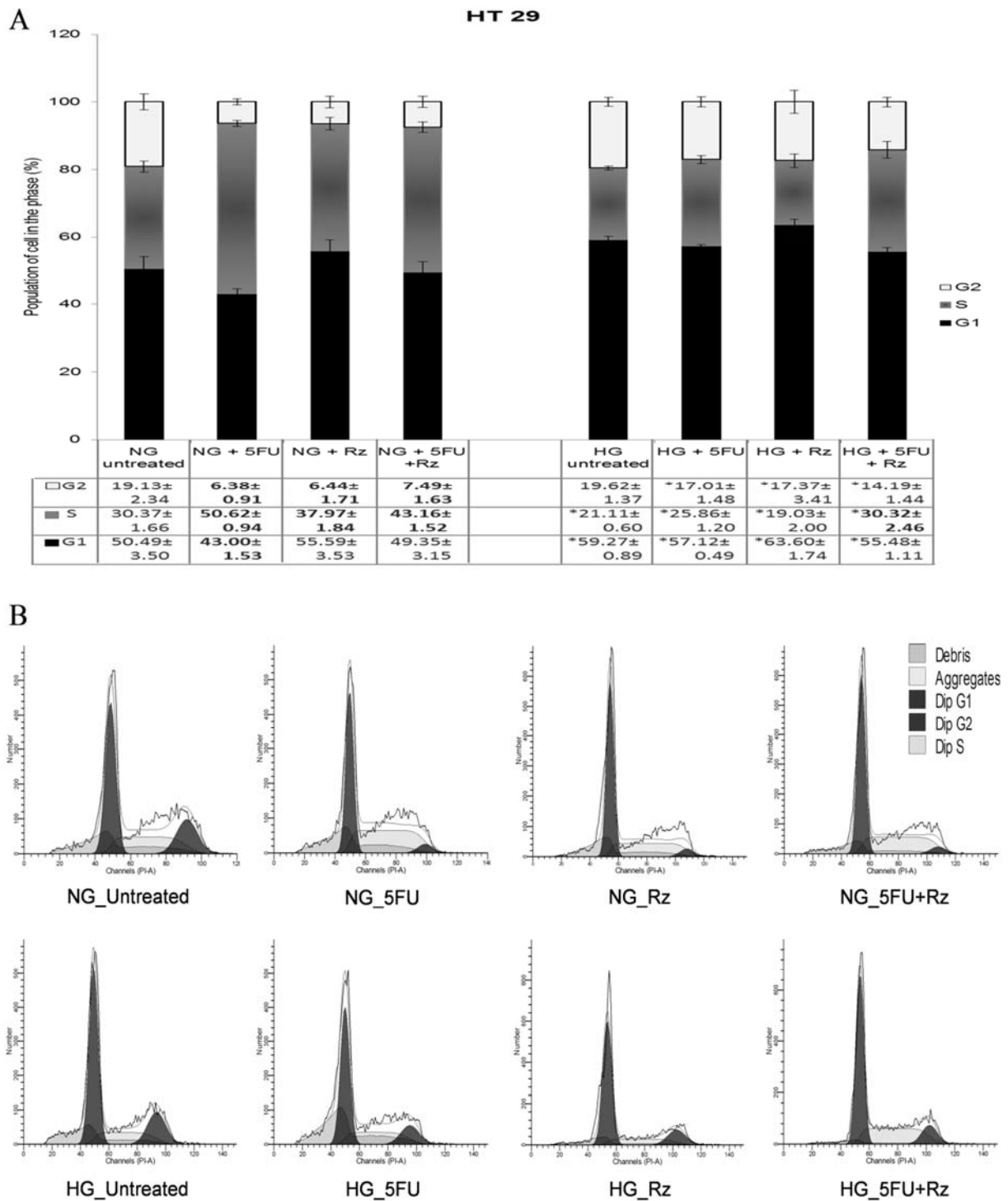


Figure 5: 5-FU pretreated cells (HT 29) were exposed to different drug treatments for 48 h incubation in the present of normal glucose (NG) or high glucose (HG). **(A)** Data are presented as mean±SEM from three independent experiments in at least three replicates. Numbers in bold are significantly different compared to the values of untreated cells, $p < 0.05$. **(B)** Representative DNA content histograms analyzed by Modfit software.

*Compared to NG group with the same treatment, $p < 0.05$.

NG, normal glucose (5.5 mM); HG, high glucose (25 mM)

Concentration of 5-FU= 0.2 µg/ml; Concentration of rosiglitazone= 0.5 µg/ml

G1: Interphase Gap1; S: Interphase Synthesis; G2: Interphase Gap2

5-FU is widely used to treat metastatic colorectal cancer. It is a uracil analog and principally works through irreversible inhibition of thymidylate synthase (TS), an enzyme responsible for the conversion of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) under normal physiological conditions (Zhang et al., 2008b). 5-FU is converted to fluorodeoxyuridine monophosphate (FdUMP) which competes with dUMP in binding to TS and thus limiting dTMP production. The dTMP depletion can lead to cytotoxicity causing cell death via thymineless death (Longley et al., 2003). Although cytotoxicity of 5-FU in colorectal cell lines have been extensively determined (Failli et al., 2011, 2013; Flis and Sławiński, 2009; Wiebke et al., 2003), the role of glucose concentrations in altering the drug cytotoxicity still need to be clarified. In the present study, we showed that 5-FU and high glucose affected the cancer cell proliferation antagonistically.

As demonstrated in the study, the cytotoxicity of 5-FU in colorectal cancer cells was diminished by HG treatment, a concentration equivalent to the serum glucose level in diabetic individuals (glucose level >200 mg/dl). Conversely, proliferative effect of high glucose could be overcome when dosage of the drug was high enough. Ma et al. (2014) speculated that a higher administration dosage is required to sustain the therapeutic effect of 5-FU for CRC patients with hyperglycemia. Unfortunately, cases of 5-FU toxicity have been reported on diabetic patients and the severity was directly related to the degree of hyperglycemia (Sadoff, 1998). It implicated that administration of 5-FU can pose a threat to developing drug toxicity if blood glucose was poorly managed. Past clinical investigations have proposed that deficiency of, either dihydropyrimidine dehydrogenase or dihydropyrimidinase, is a pharmacogenetic disorder associated with 5-FU toxicity (Milano et al., 1999; van Kuilenburg, 2004; van Kuilenburg et al., 2003), but the activities of these 5-FU catabolic

enzymes in the cancer patients with diabetes are still not fully understood.

As the mainstay of chemotherapy for colorectal cancer, some common adverse events induced by 5-FU have been reported, namely mucositis, diarrhea and myelosuppression (Vincenzi et al., 2008). 5-FU was also found to elevate blood glucose level in CRC patients (Köhne et al., 1997; Tayek and Chlebowski, 1992). A recent study even concluded that hyperglycemia is a potent complication due to 5FU-based regimen (Feng et al., 2013). To address this issue, groups of antidiabetic agents including insulin analogs, insulin sensitizers, secretagogues and incretin mimetics are currently being considered. Based on a review by García-Jiménez et al. (2016), agents that improve insulin sensitivity may reduce cancer risk rather than those that increase circulating insulin. Therefore, rosiglitazone as an insulin sensitizer should be appropriately applied in combination with 5-FU when taking cancer risk factors into consideration.

Ample evidences showed that rosiglitazone, a PPAR-gamma agonist suppressed proliferation of various cancerous cells at concentrations varying from 0.1 $\mu\text{mol/L}$ to 100 $\mu\text{mol/L}$ (Cao et al., 2009, 2015; Han and Roman, 2006; He et al., 2008; Lin et al., 2007; Zhang et al., 2008a). Similarly, in the present study, 5-FU pretreatment followed by a comparable dosage of rosiglitazone inhibited cell growth of HCT 116 and HT 29. While only one glucose concentration (16.67 mM or 11.11 mM) was used in the previous related studies (Lin et al., 2007; Miao et al., 2011; Zhang et al., 2007), this study demonstrated that the inhibitory effect of rosiglitazone was modulated by glucose levels. Activation of PPAR-gamma by rosiglitazone stimulates the expression of phosphate and tension homolog (PTEN) in human carcinoma cell lines (Han and Roman, 2006; Cao et al., 2009). When glucose level is high, the PTEN expression is decreased leading to an increased Akt activity (Liu et al., 2012; Mahimainathan et al., 2006). The deregulation of Akt signaling allows cell

survival and cell growth which may explain why the inhibitory effect of rosiglitazone was significantly ($p < 0.05$) reduced in HG culture (Figures 2C₁-C₂). There are other possible downstream regulations via a PPAR-gamma dependent signal pathway such as COX-2, MMP-7 and TIMP-1 (Miao et al., 2011).

5-FU treatment causes DNA damage due to misincorporation of FdUTP into DNA (Longley et al., 2003). While cell cycle progression is regulated by checkpoint control in the G1 or G2 phase, cycle arrests in G1 and G2 phases allow DNA repair prior to replication and mitosis respectively. In the present study, low dose of 5-FU appeared to trigger cytostasis via G1 phase arrest on HCT 116 cells but S phase arrest on HT 29 cells in NG culture. The different cell cycle response might be due to the variance in mutation status of HCT 116 (MMR-deficient/p53-proficient) and HT 29 (MMR-proficient/p53-deficient). Admittedly, MMR-deficient cell lines confer less sensitivity to 5-FU (Adamsen et al., 2011) thus, HG could diminish G1 phase arrest on 5FU-treated HCT 116 cells. The result was consistent with the increase of cell proliferation at high glucose level (Figure 2B₁). On the other hand, Hawn et al. (1995) suggested that any agent that induces DNA mispairs will lead to G2 arrest in MMR-proficient cells. The increase of HT 29 cells in G2 phase corresponded to the increase of cell proliferation (Figure 2B₂) indicating that the mismatch repair (MMR) system interacted with G2 checkpoint in response to 5-FU, especially at high glucose level for DNA repair which allow cell replication. Unlike 5-FU, low dose rosiglitazone induced S-phase arrest and this differed to the effects whereby G1-phase arrest was evident on gastric cancer (He et al., 2008), breast cancer (Zhang et al., 2008a), and liver cancer (Yu et al., 2010). In fact, rosiglitazone was also reported to cause G1-phase arrest in colorectal cancers at higher concentrations specifically 10 $\mu\text{mol/L}$ (HT 29) (Lin et al., 2007) and 25 $\mu\text{mol/L}$ (HCT 15) (Miao et al., 2011). These conflict-

ing results suggested that low dose of rosiglitazone might trigger the cells to experience genotoxic stress during DNA replication and delay their progression in a transient manner through activation of intra-S-phase checkpoint (Bartek et al., 2004). Proteins that may be involved in the intra-S-phase checkpoint are kinase Chk1, MSH2 and MCH 1. It is possible to enhance the efficacy of 5-FU if DNA synthesis can be inhibited by regulating the TS levels during the S phase of cell cycle (Subbarayan et al., 2010). The down-regulation of TS induces p53 protein expression (Liu et al., 2002) and initiates cell apoptosis consequently.

To determine the drug interaction between rosiglitazone and 5-FU, the cytotoxicity outcomes from individual and combined drug treatments were further analyzed using combination index (CI) method (Chou and Talalay, 1983, 1984). Based on CI values generated by CompuSyn software version 1.0 (data not shown), the present study revealed that high dose of rosiglitazone exerted synergistic effect on 5-FU treatment regardless of glucose levels. Indeed, it was far more effective than 5FU/metformin combination whereby the cell growth suppression occurred at a higher dosage of both drugs (Zhang et al., 2013). Addition of low dose rosiglitazone was antagonistic to 5-FU, meaning that the combined drugs had an overall effect that was less than the sum of their individual effect. The cell cycle analysis reflected a similar trend between 5-FU treatment and combined drug treatment, whereby the highest cell proportion occurred in G1 phase followed by S phase and G2 phase under NG or HG culture.

CONCLUSION

This study is the first attempt to demonstrate the influence of rosiglitazone on cytotoxicity of 5-FU under normal or high glucose condition. The present results showed that rosiglitazone had an antiproliferative effect on colorectal cancer cells via G1 or S phase arrest. Moreover, the antiproliferative effect was synergistic to 5-FU drug in the

presence of high glucose. Thus, combining rosiglitazone in 5-FU regimen may improve the therapeutic effect. Taken together, the present finding provides a better insight for the management of hyperglycemic CRC patients on 5-FU chemotherapy.

Acknowledgement

This work was supported by University of Malaya Research Grant BK025-2014.

Declaration of interest

The authors disclose no conflicts of interest.

REFERENCES

- Adamsen BL, Kravik KL, de Angelis PM. DNA damage signaling in response to 5-fluorouracil in three colorectal cancer cell lines with different mismatch repair and TP53 status. *Int J Oncol.* 2011;39:673-82.
- André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med.* 2004;350:2343-51.
- Bartek J, Lukas C, Lukas J. Checking on DNA damage in S phase. *Nat Rev Mol Cell Biol.* 2004;5:792-804.
- Cao L, Wang X, Wang Q, Xue P, Jiao X, Peng H, et al. Rosiglitazone sensitizes hepatocellular carcinoma cell lines to 5-fluorouracil antitumor activity through activation of the PPAR γ signaling pathway. *Acta Pharmacol Sin.* 2009;30:1316-22.
- Cao X, He L, Li Y. Effects of PPAR γ agonist-rosiglitazone on human retinoblastoma cell *in vitro* and *in vivo*. *Int J Clin Exp Pathol.* 2015;8:12549-56.
- Chou TC, Talalay P. Analysis of combined drug effects: a new look at a very old problem. *Trends Pharmacol Sci.* 1983;4:450-4.
- Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul.* 1984; 22:27-55.
- de Bruijn KMJ, Arends LR, Hansen BE, Leeflang S, Ruiter R, van Eijck CHJ. Systematic review and meta-analysis of the association between diabetes mellitus and incidence and mortality in breast and colorectal cancer. *Br J Surg.* 2013;100:1421-9.
- Deng L, Gui Z, Zhao L, Wang J, Shen L. Diabetes mellitus and the incidence of colorectal cancer: an updated systematic review and meta-analysis. *Dig Dis Sci.* 2012;57:1576-85.
- Failli A, Consolini R, Legitimo A, Orsini G, Romani A, Spisni R, et al. Evaluation of *in vitro* cytotoxicity of oxaliplatin and 5-fluorouracil in human colon cancer cell lines: combination versus sequential exposure. *J Biol Regul Homeost Agents.* 2011;25:575-88.
- Failli A, Legitimo A, Orsini G, Castagna M, Spisni R, Miccoli P, et al. Antiproliferative effects of 5-fluorouracil and oxaliplatin in colon cancer cell lines: comparison of three different cytotoxicity assays. *J Biol Regul Homeost Agents.* 2013;27:275-84.
- Feng JP, Yuan XL, Li M, Fang J, Xie T, Zhou Y, et al. Secondary diabetes associated with 5-fluorouracil-based chemotherapy regimens in non-diabetic patients with colorectal cancer: results from a single-centre cohort study. *Color Dis.* 2013;15:27-33.
- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer.* 2015;136:E359-86.
- Flis S, Splawiński J. Inhibitory effects of 5-fluorouracil and oxaliplatin on human colorectal cancer cell survival are synergistically enhanced by sulindac sulfide. *Anticancer Res.* 2009;29:435-41.
- García-Jiménez C, Gutiérrez-Salmerón M, Chocarro-Calvo A, García-Martínez JM, Castaño A, de la Vieja A. From obesity to diabetes and cancer: epidemiological links and role of therapies. *Br J Cancer.* 2016; 114:716-22.
- Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes care.* 2010;33: 1674-85.
- Goodwin RA, Asmis TR. Overview of systemic therapy for colorectal cancer. *Clin Colon Rectal Surg.* 2009;22:251-6.
- Han S, Roman J. Rosiglitazone suppresses human lung carcinoma cell growth through PPAR γ -dependent and PPAR γ -independent signal pathways. *Mol Cancer Ther.* 2006;5:430-7.
- Hawn MT, Umar A, Carethers JM, Marra G, Kunkel TA, Boland CR, et al. Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. *Cancer Res.* 1995;55:3721-5.

- He Q, Pang R, Song X, Chen J, Chen H, Chen B, et al. Rosiglitazone suppresses the growth and invasiveness of SGC-7901 gastric cancer cells and angiogenesis *in vitro* via PPAR γ dependent and independent mechanisms. *PPAR Res.* 2008;2008:649808.
- Kashif M, Andersson C, Hassan S, Karlsson H, Senkowski W, Fryknäs M, et al. *In vitro* discovery of promising anti-cancer drug combinations using iterative maximisation of a therapeutic index. *Sci Rep.* 2015;5:14118.
- Köhne CH, Harstrick A, Hiddemann W, Schöffski P, Wilke H, Bokemeyer C, et al. Modulation of 5-fluorouracil with methotrexate and low-dose N-(phosphonacetyl)-L-aspartate in patients with advanced colorectal cancer. Results of a phase II study. *Eur J Cancer.* 1997;33:1896-9.
- Krämer HU, Schöttker B, Raum E, Brenner H. Type 2 diabetes mellitus and colorectal cancer: meta-analysis on sex-specific differences. *Eur J Cancer.* 2012;48:1269-82.
- Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: A meta-analysis. *J Natl Cancer Inst.* 2005;97:1679-87.
- Lin MS, Chen WC, Bai X, Wang YD. Activation of peroxisome proliferator-activated receptor gamma inhibits cell growth via apoptosis and arrest of the cell cycle in human colorectal cancer. *J Dig Dis.* 2007;8:82-8.
- Liu J, Schmitz JC, Lin X, Tai N, Yan W, Farrell M, et al. Thymidylate synthase as a translational regulator of cellular gene expression. *Biochim Biophys Acta-Mol Basis Dis.* 2002;1587:174-82.
- Liu L, Hu X, Cai GY, Lv Y, Zhuo L, Gao JJ, et al. High glucose-induced hypertrophy of mesangial cells is reversed by connexin43 overexpression via PTEN/Akt/ mTOR signaling. *Nephrol Dial Transplant.* 2012;27: 90-100.
- Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer.* 2003;3:330-8.
- Luo W, Cao Y, Liao C, Gao F. Diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. *Color Dis.* 2012;14: 1307-12.
- Ma YS, Yang IP, Tsai HL, Huang CW, Juo SHH, Wang JY. High glucose modulates antiproliferative effect and cytotoxicity of 5-fluorouracil in human colon cancer cells. *DNA Cell Biol.* 2014;33:64-72.
- Mahimainathan L, Das F, Venkatesan B, Choudhury GG. Mesangial cell hypertrophy by high glucose is mediated by downregulation of the tumor suppressor PTEN. *Diabetes.* 2006;55:2115-25.
- Masur K, Vetter C, Hinz A, Tomas N, Henrich H, Niggemann B, et al. Diabetogenic glucose and insulin concentrations modulate transcriptome and protein levels involved in tumour cell migration, adhesion and proliferation. *Br J Cancer.* 2011;104:345-52.
- Miao R, Xu TAO, Liu L, Wang M, Jiang Y, Li J, et al. Rosiglitazone and retinoic acid inhibit proliferation and induce apoptosis in the HCT-15 human colorectal cancer cell line. *Exp Ther Med.* 2011;2:413-7.
- Milano G, Etienne MC, Pierrefite V, Barberi-Heyob M, Deporte-Fety R, Renee N. Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. *Br J Cancer.* 1999;79:627-30.
- Mills KT, Bellows CF, Hoffman AE, Kelly TN, Gagliardi G. Diabetes mellitus and colorectal cancer prognosis: a meta-analysis. *Dis Colon Rectum.* 2013; 56:1304-19.
- Monami M, Dicembrini I, Mannucci E. Thiazolidinediones and cancer: results of a meta-analysis of randomized clinical trials. *Acta Diabetol.* 2014;51:91-101.
- Sadoff L. Overwhelming 5-fluorouracil toxicity in patients whose diabetes is poorly controlled. *Am J Clin Oncol.* 1998;21:605-7.
- Subbarayan PR, Lee K, Ardalán B. Arsenic trioxide suppresses thymidylate synthase in 5-FU-resistant colorectal cancer cell line HT29 *in vitro* re-sensitizing cells to 5-FU. *Anticancer Res.* 2010;30:1157-62.
- Tayek JA, Chlebowski RT. Metabolic response to chemotherapy in colon cancer patients. *JPEN J Parenter Enter Nutr.* 1992;16:65S-71S.
- Tomas NM, Masur K, Piecha JC, Niggemann B, Zänker KS. Akt and phospholipase C γ are involved in the regulation of growth and migration of MDA-MB-468 breast cancer and SW480 colon cancer cells when cultured with diabetogenic levels of glucose and insulin. *BMC Res Notes.* 2012;5:1-7.
- van Kuilenburg ABP. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer.* 2004;40:939-50.
- van Kuilenburg ABP, Meinsma R, Zonnenberg BA, Zoetekouw L, Baas F, Matsuda K, et al. Dihydropyrimidinase deficiency and severe 5-fluorouracil toxicity. *Clin Cancer Res.* 2003;9:4363-7.

- vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009;324:1029-33.
- Vincenzi B, Schiavon G, Pantano F, Santini D, Tonini G. Predictive factors for chemotherapy-related toxic effects in patients with colorectal cancer. *Nat Clin Pr Oncol*. 2008;5:455-65.
- Wiebke EA, Grieshop NA, Loehrer PJ, Eckert GJ, Sidner RA. Antitumor effects of 5-fluorouracil on human colon cancer cell lines: antagonism by levamisole. *J Surg Res*. 2003;111:63-9.
- Yki-Järvinen H. Thiazolidinediones. *N Engl J Med*. 2004;351:1106-18.
- Yu J, Shen B, Chu ES, Teoh N, Cheung KF, Wu CW, et al. Inhibitory role of peroxisome proliferator-activated receptor gamma in hepatocarcinogenesis in mice and *in vitro*. *Hepatology*. 2010;51:2008-19.
- Zhang YQ, Tang XQ, Sun L, Dong L, Qin Y, Liu HQ, et al. Rosiglitazone enhances fluorouracil-induced apoptosis of HT-29 cells by activating peroxisome proliferator-activated receptor gamma. *World J Gastroenterol*. 2007;13:1534-40.
- Zhang T, Zhang Q, Chen D, Jiang J, Zhou Q. Growth inhibition of human breast cancer cell line MDA-MB-231 by rosiglitazone through activation of PPAR γ . *Chinese J Clin Oncol*. 2008a;5:407-12.
- Zhang N, Yin Y, Xu SJ, Chen WS. 5-fluorouracil: mechanisms of resistance and reversal strategies. *Molecules*. 2008b;13:1551-69.
- Zhang Y, Guan M, Zheng Z, Zhang Q, Gao F, Xue Y. Effects of metformin on CD133+ colorectal cancer cells in diabetic patients. *PLoS One*. 2013;8:e81264.