

EFFECT OF RADIATION ON THE VIABILITY OF HepG2 CANCER CELL LINE TARGETED WITH DIFFERENT AMOUNT OF RADIOSENSITIZER

(Kesan Sinaran Terhadap Kemandirian Sel Kanser HepG2 Yang Disasar Dengan Pelbagai Isipadu Pemeka Sinaran)

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Abstract

Radiosensitizer (RS) were applied prior to radiation therapy to increase the therapeutic efficacy. This is due to some cancer cells were resistance toward radiation by producing large amount of antioxidant enzyme to scavenges the free radicals. The amount of RS added prior to irradiation played an important role to increase the efficacy of treatment but peroxide may cause unnecessary stress to the cancer cells before the treatment of irradiation. In this paper, different amount of RS were tested to evaluate the optimum amount of RS to avoid over-stress to the cancer cells while showing radiosensitizing effect by comparing to sample without RS. RS with different concentration started from 5% to 50% are added to hepatocellular carcinoma (HepG2) cancer cell line prior to 2Gy of fractionated dose. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 24 hours after irradiation. In this study it is observed that the concentration of RS range between 5-20% did not cause reduction on the viability percentage of the studied HepG2 cells whereas 25-50% caused significant reduction on cell viability. The radiosensitizing effect of RS at 20% concentration exhibits 18.68% more cell death compared to treatment with radiation alone. It is therefore suggested that 20% is the optimum amount of RS which able to enhance the radiosensitizing ability without causing toxicity to the cell, thus induced more cancer cell death during radiotherapy.

Keywords: Radiosensitizer, hyaluronan, radiotherapy, HepG2, MTT assay

Abstrak

Pemeka Sinaran (RS) diaplikasi sebelum terapi sinaran untuk meningkatkan keberkesanan rawatan kanser melalui kaedah sinaran. Pemeka sinaran digunakan kerana sebahagian sel kanser bersifat rintang terhadap sinaran yang disebabkan penghasilan enzim antioksidan yang banyak lalu menghalang radikal bebas mendekati sel kanser. Penambahan jumlah RS sebelum penyinaran memainkan peranan penting untuk meningkatkan keberkesanan rawatan radioterapi tetapi peroksida boleh membawa tekanan yang keterlaluan ke atas sel sebelum rawatan penyinaran. Dalam kajian ini, pelbagai kepekatan RS telah digunakan untuk menentukan kuantiti optimum bagi mengelakkan kesan keterlaluan RS ke atas sel kanser. Kepekatan RS dalam julat 5% hingga 50% disasarkan ke atas sel kanser HepG2 sebelum dos sebanyak 2Gy diberikan. Kemandirian sel diuji 24 jam selepas penyinaran dengan 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide asai MTT. Kajian ini mendapati bahawa kuantiti RS dari 5% ke 20% tanpa disinari menunjukkan tiada pengurangan dari segi peratusan kemandirian sel HepG2 manakala RS 25-50% menunjukkan pengurangan peratusan kemandirian sel HepG2 yang ketara. Kesan kepekatan RS pada 20% berupaya mengaruh 18.68% lebih kematian sel berbanding dengan rawatan sinaran tanpa RS. Kesimpulannya, kuantiti RS pada 20% adalah optimum dalam meningkatkan kesan kepekatan sinaran tanpa ketoksikan ke atas sel dan mengaruh lebih banyak kematian sel kanser ketika radioterapi dijalankan.

Kata kunci: Pemeka sinaran, hyaluronan, radioterapi, HepG2, asai MTT

Introduction

Many attempts for cancer treatment have been conducted using different approach, one of it were radiation therapy. But the effects of radiation could damage the healthy tissue while damaging the cancerous tumor, thus researches discovered that the fractionated radiation dose through 5Rs of radiobiology which is repair, reassortment, reoxygenation, repopulation and radiosensitivity played important role for protecting the normal cells while enhancing the efficacy of this therapy [1]. Some cancerous cells are resistance to radiation where the antioxidant enzymes that were produced scavenged the free radical [2]. Through the use of peroxide based radiosensitizer, the antioxidant enzyme could be regulated and thus increase the sensitivity of cancerous cell toward radiation [3] while the oxygen produced are theoretically able to combine with free radical in which producing another toxic combination [4]. Radiosensitizer announced by Ogawa et al [5] were combination of hydrogen peroxide (HP) and sodium hyaluronate (HA) which is able to sensitized the cancerous cell and on the other hand, re-oxygenate the area of cancer cell thus it further damaging the oxidized cells. An in-vitro investigation on HepG2 cancer cell line showed the amount of RS might cause cytotoxicity before the actual treatment of radiotherapy. Thus it is necessary to further the research by looking the optimum acceptance amount of RS.

Materials and Methods

Preparation of HepG2 Cell Culture

HepG2 cell line were cultured using complete medium which is made up from 174mL of Eagle's Minimum Essential Medium (EMEM), L-glutamin, 2.2g sodium biocarbonate and growth serum that composed of four different agent which is 20mL of fetal bovine serum, 2mL of sodium pyruvate, 2mL of non-essential amino acid and 2mL of penicillin streptomycin solution that is purchased from Gibco. HepG2 cell line from American Type Culture Collection (ATCC) is thawed from frozen and incubated in T25 culture flask. The culture is carried out under aseptic practice to minimize and avoid contamination of cell culture. During subculture, the cells were washed with phosphate buffer saline (PBS) before trypsinization for 3 minutes. After incubation of 3 minute, the detachment of cells is confirmed under inverted microscope then complete medium is added immediately. Calculation of cell density using haemocytometer together with trypan blue dye and 5×10^5 cells/mL is calculated and seeded into 96 well plate.

Synthesis of Hyaluronate and Peroxide based Radiosensitizer

Sodium hyaluronate (HA) was in powder form when bought from Sigma, it is added with ionized water to obtained 1% w/v HA whereas for hydrogen peroxide (HP), it is bought in a formulation of 30% w/v and diluted with ionized water to obtained 3% v/v HP. HA was prepared one day before the treatment to allowed the powder HA to distribute evenly in ionized water but for HP is prepared on the day of treatment to avoid early oxidation of HP. Synthesis of RS begins with adding 3% v/v hydrogen peroxide to 1% w/v sodium hyaluronate at ratio of 1:5. It is prepared prior to distribution to every well.

Treatment of Radiosensitizer towards HepG2 Cell Viability

Amount of RS added were 10uL to 100uL which is 5% to 50% of the total volume content within each well. After RS is added, the 96 well plate is covered and transported carefully for irradiation dose at 2Gy using a Co-60 gamma irradiator (Gamma cell) and incubated immediately. Cell viability of HepG2 cancer cell line is evaluated 24 hours after irradiation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [6]. MTT solution is prepared by dissolving MTT powder in PBS to obtained 3mg/ml concentration and 30uL is used in each well. Formazan crystal is allowed to form when treated cell culture is incubated 4 hours. This formazan crystal is proportional to the viable cell survived from the treatment. The crystal is dissolved in 150uL of dimethyl sulfoxide (DMSO) and the optical absorbance is analyzed at 570nm using BIO-RAD xMark™ microplate spectrophotometer [7].

Results and Discussion

Effect of RS towards HepG2 Cell Viability

Figure 1 show cell viability of both irradiated (IRR) and non-irradiated (NO IRR) HepG2 cancer cells treated with different percentage of RS towards complete medium. RS at 50% is the maximum amount used protocol for MTT assay while 0% RS represent the control of the whole experiment. RS range from 5% to 20% (10uL-40uL) showed higher viability in non-irradiated sample compared to control sample. It is proven that at these volumes no damage

were observed or the HepG2 cells were not affected by the RS. Whereas starting from 25% to 50%, the percentage of cell viability dropped dramatically below 60% for the non-irradiated sample. It is suggested that these volumes have caused toxicity to the sample and the amount of RS must be well-controlled in order to avoid unnecessary damage towards the cells before the radiation treatment. This is due to the normal cells will be damaged at the same time when RS is introduced.

Comparison between RS with Irradiation and without Irradiation

Comparison between the curve of NO IRR (blue line) and IRR (red line), 15% RS and below did not shows much damage when irradiated. This is suggesting that the amount RS is tolerable by HepG2 cells before irradiation but does not show much damage after irradiation. For the RS with amount 25% and above, the cell viability of RS alone (blue line) shows very low viability, this suggests that the amount of RS caused significant damage to cell before irradiation in which this amount of RS is not considerable due to its cytotoxicity to cells, thus the cell viability for this range of RS is not valid as the treatment of radiotherapy due to majority of cancer cells were damaged before irradiation. RS at 20% shows no toxicity towards cell viability but after irradiation caused more reduction in cell viability compared with irradiation alone. Therefore, RS will be beneficial for radiation treatment when it is controlled at 20%.

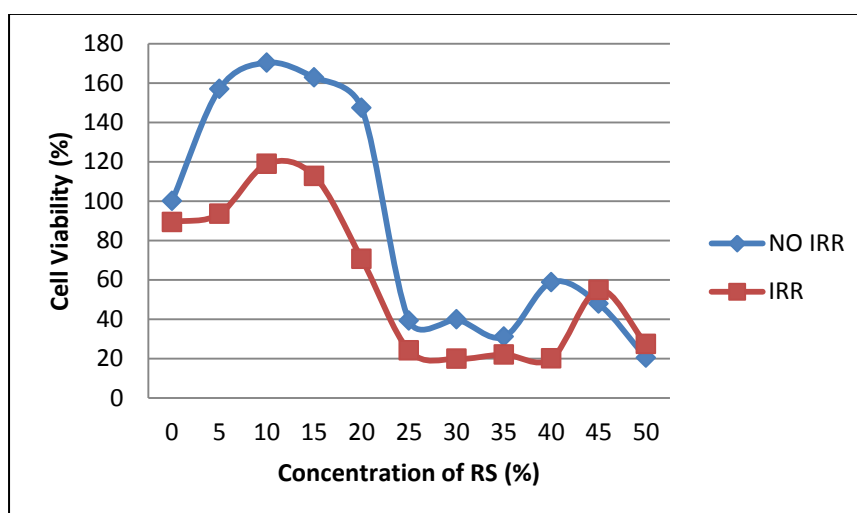


Figure 1. Cell viability of both irradiated (red line) and non-irradiated (blue line) of HepG2 cancer cells treated with different concentration of RS.

Effect of 20% RS towards HepG2 Cell Viability

Figure 2 shows detailed result of 20% concentration of RS with respect to cell viability at control (NO RS NO IRR), RS alone (RS NO IRR), radiation alone (NO RS IRR) and both RS and irradiation (RS IRR) samples. NO RS NO IRR represents the control sample with 100% of cell viability, which is a fixed percentage for comparison between other samples. For the sample of RS NO IRR, which is the RS alone without any radiation treatment is for the purpose of whether it is cytotoxicity toward the HepG2 cell line before irradiation. In the graph, it showed that the cell viability is exceeding 100%, which is 147.26%. Therefore it is suggested 20% of RS does not caused cytotoxicity.

From the graph of NO RS IRR, irradiation alone or represents the normal radiotherapy, shows the percentage of cell viability is reduced by 10.65% which remain 89.35% of viable cells. By adding RS prior to irradiation, the percentage of cell viability reduced 29.33%, remain 70.67% viable, which is 18.68% more damage compared to irradiation alone. Thus, RS with irradiation at this concentration caused more reduction in cell viability.

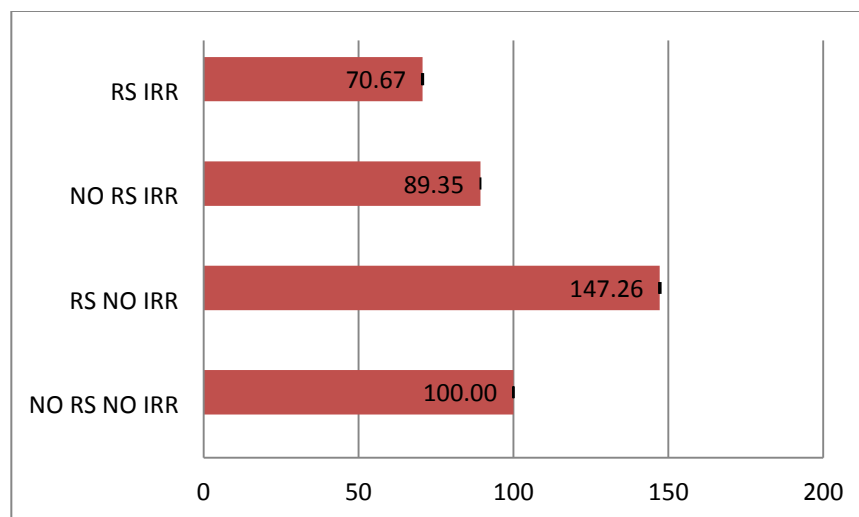


Figure 2. Cell viability of HepG2 cells evaluated after 24 hours after with and without irradiation and with and without prior addition of RS at 20% concentration

Conclusion

RS at 20% shows no toxicity towards HepG2 cell line but addition of RS prior to irradiation caused reduction in cell viability. Amount of RS below or above 20% shows either no effect or caused cytotoxic to HepG2 cell line before irradiation. Therefore controlling an optimum acceptance amount of RS is very crucial and important so that no unnecessary stress or damage occurred to non-cancerous cell or tissue during the radiation treatment.

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