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The Effect of Sn(IV) Chlorin e6 on HepG2 Cancer Cell Lines

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Abstract: This research investigated the effect of Sn(IV) chlorin e6 dichloride trisodium salt photosensitizer on the viability of HepG2 cancer cell lines in vitro. The effect of light on the viability of cells without the photosensitizer and the toxicity of the photosensitizer in the absence of light were examined in this research. No toxic effects with the absence of light were found and no photodamage effect on the cells without the presence of photosensitizer. The effect of different concentrations of the photosensitizer with fixed light dose of 70 J/cm² on the viability of HepG2 cancer cells were performed. Then, two concentrations 25 and 30 µg/ml were investigated at different light doses 60-100 J/cm². The effect of the photosensitizer on the viability of HepG2 at different light doses and different concentrations was found to have low viability over concentrations of 25 µg/ml. This could be due to that the photosensitizer reached a saturated status after this concentration.

Key words: PDT; Sn(IV) chlorin e6; liver cancer; phototoxicity; viability assay

1. INTRODUCTION

Liver cancer (hepatocellular carcinoma) or hepatoma Liver cancer is the fifth most common cancer in the world. Liver cancer kills almost all the patients who have it within a year. In 1990 there were 430,000 new cases of liver cancer over the world as was estimated by the World Health Organization and a similar number of patient died as a result of this disease (Jianhua and Xinhong, 1998). The most cases were in East Asia and sub-saharan Africa (Mozambique and South Africa).

As a promising treatment methodology photodynamic therapy PDT was used to treat such cancers. PDT requires a photosensitizer and light with proper wavelength with presence of oxygen. When the photosensitizer is exposed to light its molecules will be excited and by decaying through the triplet state there will be a generation of singlet oxygen (1O_2) and reactive oxygen species (ROS) which are toxic to cells and tissue (Ding, et al., 2004).

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The effect of Phthalocyanine and metal-free hexadeca-carboxy phthalocyanine photosensitizer on HepG2 cancer cells were examined by Chi-Fung and coworkers. There results exhibited that HepG2 was resistant to the phototoxic effect of these two photosensitizers with concentrations up to 4 μ M and light dose of 40 J/cm² (Chi-Fung, et al., 2004). While other studies on amphiphilic pthalocyanines found to be potent against HepG2 cancer cells (Lo, et al., 2004). Yow and coworkers investigate the effect of 5-ALA photosensitizer on HepG2 liver cancer cell lines (Yow, Wong, Huang, and Ho, 2007). Using PAD-S31 photosensitizer in PDT with concentration of 100 μ g/ml, 2 hours incubation time and 20 J/cm² of light dose (Date, et al., 2004). In this study investigating the effect of PDT on the viability of HepG2 hepatoma liver cancer cell lines using Sn(IV) chlorin e6 dichloride trisodium in will be performed.

2. METHODOLOGY

2.1 Chemicals

Sn(IV) chlorin e6 dichloride Trisodium salt (Frontier Scientific, USA), was dissolved in PBS with a concentration 1mg/ml and kept frozen before use.

2.2 Cell lines

HepG2 human liver hepatocellular carcinoma cell lines (ATCC, USA) were used in this research. Cells were cultured in EMEM supplemented with 10% Fetal bovine serum (FBS) (Gibco, Malaysia). These cells were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO₂.

2.3 Cell viability assay

MTT salt (Sigma, Malaysia) was used to determine the viability of the cell lines throughout this research. MTT was dissolved in PBS with concentration of 5 mg/ml directly before use. 100 μ l medium containing 20 μ l MTT solution was added to each well and the microplate was incubated at 37°C, 5% CO₂ for 4 hours. At the end of incubation period the medium was discarded gently and replaced by 100 μ l of DMSO. The plate was shaken on the microplate shaker to dissolve formazan. Absorbance was recorded at the 570 nm on a microplate reader with 620 nm as reference wavelength.

2.4 PDT treatment

Fresh medium containing HepG2 cells were added into 96 well plates at 2 x 10⁴ cells per well and incubated in the same conditions overnight to allow attachment to the bottom. Then, the cells were treated with different concentrations of Sn(IV) chlorine e6 (15, 20, 25 and 30 μ g/ml) and incubated for 6 h. the first 8 well contained fresh medium alone and used as blank the second 8 wells contained cells without photosensitizer and were used as a controls. The cells of both PDT and control groups were irradiated with 635 nm light from LLC-7 high pressure spherical Xenon lamp (Lambda Scientific, North Adelaina, USA) using light red filter (LFord, England) cut the wavelength less than 550 nm with a light dosage of 70 J/cm². After light treatment the cells were incubated for 24 h before the cell viability was determined by the MTT assay.

2.5 Light Toxicity

The above discribed process was followed to examine the effect of light on the viability of HepG2 cancer cells. Different light doses (60, 70, 80, 90, 100 J/cm²) were applied on the cells incubated in 96-well plate without the photosensitizer.

2.6 Dark Toxicity

The cells were incubated in dark in 96-well plates with different concentrations of the Sn(IV) chlorin e6 photosensitizer (10, 15, 20, 25 and 30 $\mu\text{g/ml}$) and was not treated with light.

3. RESULTS AND DISCUSSION

The light toxicity on HepG2 cancer cells was examined at different light doses with the absence of the photosensitizer as shown in Figure 1. The viability of the cells was more than 95%. These data show a lack of phototoxicity in the irradiated cells because no differences were observed compared with the non irradiated cells. This indicates that the delivered light did not cause any damage to the cells.

The dark toxicity of Sn(IV) chlorin e6 was examined and represented in Figure 2. There was a lack of toxicity effect of the photosensitizer in dark which is necessary for PDT. The results exhibited slightly differences in the viability of the cells with and without the presence of the photosensitizer in the absence of light. This result indicates that this Sn(IV) chlorin e6 is not toxic with absence of light at the indicated concentrations.

Figure 3 shows the effect of Sn(IV) chlorin e6 on the viability of HepG2 cancer cells at different concentrations of the photosensitizer, fixed light dose of 70 J/cm^2 , and 6 hours of incubation time. There was a gradual decreasing of the viability with proportional to the Sn(IV) chlorin e6 concentrations. A small difference can be observed between 25 and 30 $\mu\text{g/ml}$. The survival rates of HepG2 cells were from 71% \pm 5.1% to 52% \pm 2.5% with Sn(IV) chlorin e6 concentrations from 15 to 30 $\mu\text{g/ml}$. There was steep decreasing in the viability of the cells from 15 to 25 $\mu\text{g/ml}$ while no significant difference was observed from 25 to 30 $\mu\text{g/ml}$. So these result suggested that HepG2 cellular uptake of Sn(IV) chlorin e6 increased with the incubation of the photosensitizer at concentrations from 15 to 25 $\mu\text{g/ml}$ and then became saturated at higher concentrations. Accordingly, no significant increasing in the generation of singlet oxygen or ROS which cause the damage the cells. This result is important in clinical PDT in order to optimize the dose of Sn(IV) chlorin e6.

Figure 4 represents the viability of HepG2 cancer cell lines as a function of light dose at two concentrations 25 and 30 $\mu\text{g/ml}$ with 6 hours incubation time. There was a gradual decreasing of the viability by the increasing of light dose but this decreasing of viability is not steep. The lowest viability was about 52% at light dose of 100 J/cm^2 and 30 $\mu\text{g/ml}$ concentration. Comparing the effect of two concentrations it can be seen from Fig. 4 that there is no significant difference between the effect of the two concentrations. This could support our suggestion on the basis of saturation.

Enough energy is necessary to complete the excitation process of the photosensitizer molecules. If there is not enough energy the number of excited molecules will be reduced which results in a lack of the biological effects. This illustrates that the survival rate will decrease as the light dose increases. This increasing could be explained on the basis of that the increase of light dose increases the energy which resulted in more absorption of photons by molecules ,subsequently more generation of singlet oxygen and ROS then more cell damage. The results are with good agreement with this suggestion.

The effect of Sn(IV) chlorin e6 on the viability of HepG2 was less than effect of other photosensitizers such as 5-ALA and PAD-S31 which reached 80% of phototoxicity, while this photosensitizer did not exceed 50% at the indicated concentrations and doses. This could be due to the less phosphorescence which are the most important in PDT compared with fluorescence for the excited molecules. Also since the photobleaching of the photosensitizer reduces its absorption of light (Ishii, et al., 2008), it could be an important factor in the lack of phototoxicity of Sn(IV) chlorin e6.

4. CONCLUSION

In conclusion, Sn(IV) chlorin was found to be non toxic in dark and cause damage to HepG2 liver cancer cells. The PDT damage to HepG2 cells increased with increasing of the concentration of the photosensitizer and then plateaued at concentrations more than 25 µg/ml. The cell killing was proportionally to light doses of the irradiated light. More experiments may be performed in future to investigate more characterizations of this photosensitizer and its effects in PDT on other cancer cell lines.

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FIGURES

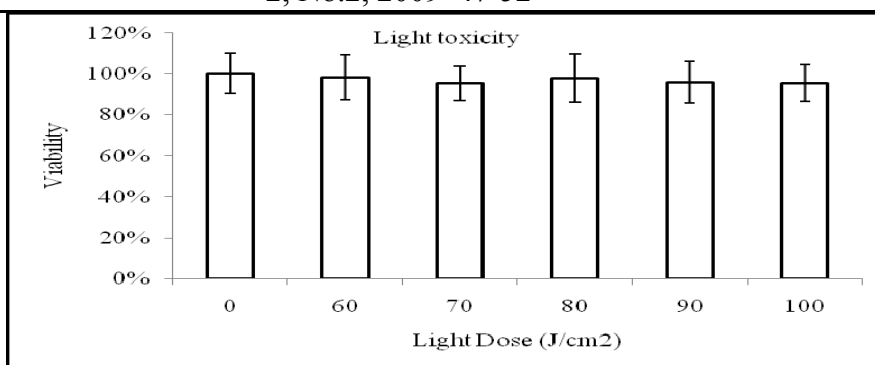


Fig. 1: The viability of HepG2 liver cancer cell lines after illuminating by different doses of light with the absence of the photosensitizer

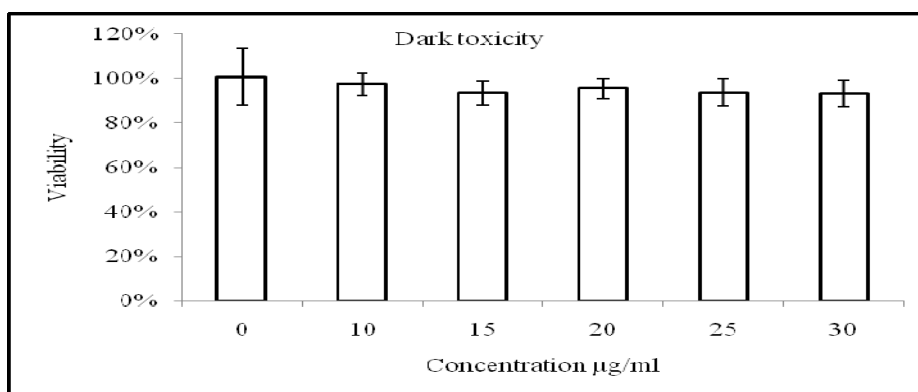


Fig. 2: The viability of HepG2 liver cancer cell lines after 12 hours of incubation time without exposing to light

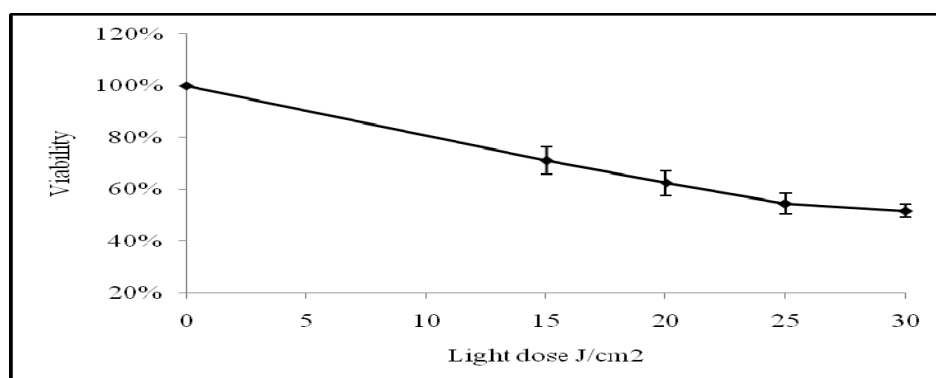


Fig. 3: The viability of HepG2 cancer cell lines in different concentrations of the photosensitizer with fixed light dose of 70 J/cm²

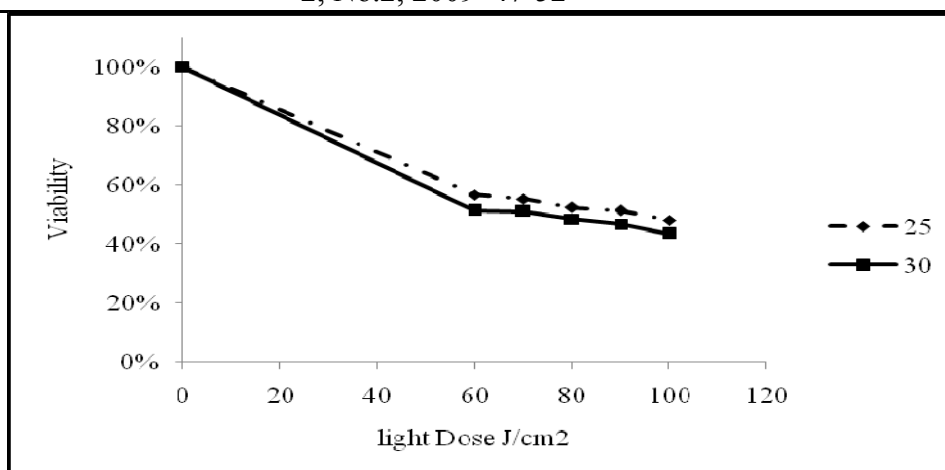


Fig. 4: The viability of HepG2 cancer cells with two photosensitiser concentrations and different light doses