Cytotoxicity Evaluation of QO_2 Against UVB Induced B164A5 Melanoma Cell Lines

AHMAD ROHI BIN GHAZALI, CHAN KAM SOON & NORAISAH AKBAR ALI

ABSTRACT

Skin cancer is one of the most common cancers worldwide. The current incidence and mortality of skin cancer and its chemotherapy resistance have pushed researchers to look for alternative chemotherapeutic strategy. Quantum Water (QO_2) is a local water product made of 100 % reverse osmosis water boosted with energy using a special quantum entanglement technology. The objective of our study was to evaluate the cytotoxicity of QO_2 by the MTT assay against UVB induced B164A5 melanoma cell lines. In the MTT assay, menadione was used as positive control, for the treatment group, the cells were treated with 5, 10, 20, 40 and 50 % (v/v) of QO_2 and the untreated cells were regarded as the negative control. Based on the MTT results, menadione showed cytotoxicity with IC_{50} of 0.03 ± 0.002 mM and QO_2 showed cytotoxicity at the lower range of concentrations with two IC_{50} which were 2.5 ± 0.08 and 6.5 ± 0.15 % (v/v). High cell proliferation was observed at the three consecutive QO_2 concentrations which were 10, 20 and 40 % (v/v) with the cell viability of 128.9, 132.0 and 103.0 % respectively. QO_2 at 10 and 20 % (v/v) showed significant ($p<0.05$) high cell viability than the negative control. In conclusion, QO_2 has cytotoxic effects on the treated cells at the lower range of concentrations but no cytotoxicity at the higher concentrations. QO_2 demonstrated biphasic effects on the treated cells as it can be postulated to be a pro-oxidant and antioxidant at low and high concentrations respectively.

Keywords: skin cancer, QO_2, cytotoxicity, biphasic effects

ABSTRAK

Kanser kulit merupakan salah satu kanser yang kerap dihidapi di seluruh dunia. Corak insiden dan mortaliti pada masa kini dan kerintangan rawatan kemoterapi telah mendorong para penyelidik untuk mengkaji strategi kemoterapeutik alternatif. *Quantum Water* (QO_2) merupakan produk air tempatan yang dihasilkan dari 100 % air osmosis berbalik yang dikayakan dengan tenaga melalui teknologi *quantum entanglement*. Objektif kajian ini adalah untuk mengkaji sitotoksisiti QO_2 ke atas sel selanjar B164A5 aruhan UVB melalui asai MTT. Dalam asai MTT, menadione telah digunakan sebagai kawalan positif, manakala untuk kumpulan rawatan, sel telah dirawat dengan 5, 10, 20, 40 and 50 % (v/v) QO2. Sel tanpa rawatan dijadikan sebagai kawalan negatif. Berdasarkan keputusan asai MTT, menadione telah menunjukkan sitotoksisiti dengan IC_{50} 0.03 ± 0.002 mM . QO_2 menunjukkan sitotoksisiti pada lingkungan kepekatan yang rendah dengan dua IC_{50} iaitu 2.5 ± 0.08 dan 6.5 ± 0.15 % (v/v). Kadar proliferasi yang tinggi didapati pada tiga kepekatan berterusan iaitu 10, 20 dan 40 % (v/v) dengan viabiliti sel 128.9, 132.0 dan 103.0 %. QO_2 pada kepekatan 10 dan 20 % (v/v) menunjukkan viabiliti sel yang tinggi secara signifikan ($p<0.05$) berbanding dengan kawalan negatif. Kesimpulannya, QO_2 mempunai kesan sitotoksik terhadap sel yang dirawat pada lingkungan kepekatan rendah tetapi tiada sitotoksisiti pada kepekatan tinggi. QO_2 menunjukkan kesan bi-fasa pada sel yang dirawat dan ini boleh dihipotesiskan QO_2 berkemungkinan bertindak pro-oksidan pada kepekatan rendah dan antioksidan pada kepekatan tinggi.

Kata kunci : kanser kulit, QO_2, sitotoksisiti, kesan bi-fasa

INTRODUCTION

Skin cancer can be divided into melanoma and non-melanoma while non-melanoma skin cancer is further divided into basal cell carcinoma and squamous cell carcinoma. Melanoma skin cancer has a lower incidence as compared to non-melanoma skin cancer but it has the highest mortality rate (Penta et al. 2018). Besides, melanoma skin cancer is more aggressive and high metastatic than the non-melanoma skin cancer (Strickland et al. 2015; Wang et al. 2017).

International Agency for Research on Cancer (2018) has reported that melanoma skin cancer is the 19th most common cancer in men and women whereas the non-melanoma is ranked at the 5th place. Ultraviolet (UV) radiation exposure, genetic factors, childhood's sunburn and pigment

characteristics such as colour of skin, hair and pupil, are regarded as the contributing risk factors towards skin cancer (Gandini et al. 2011). Family history and the present of melanocytic naevi can increase the melanoma skin cancer risk too (Berwick et al. 2009).

UVB is more carcinogenic by contrast to UVA. Chronic UV radiation exposure can result in oxidative damage, cell cycle alteration, DNA chain breaks, mutation, base modification, abasic sites and photoproducts such as cyclopyrimidine dimers and 6-4 photoproducts (D'Orazio 2013). Besides, UV radiation can cause tremendously production of reactive oxygen species (ROS) such as superoxides, hydrogen peroxides and hydroxyl radicals. These free radicals production beyond the antioxidant defense system will result in the oxidative damage to DNA, protein and lipids. DNA mutation and carcinogenesis are the ultimate effects following these oxidative damages (De Jager et al. 2017; D'Orazio 2013).

As for the present study, Quantum Water (QO_2) has been studied for its chemotherapeutic potential. QO_2 is a product made of 100 % reverse osmosis water and it is manufactured as a sanitizer and deodorant too. Using a particular quantum entanglement technology, the reverse osmosis water's oxygen molecule is also impregnated with boosted energy. Our study was then carried out to investigate the cytotoxicity of the QO_2 against the UVB induced B164A5 melanoma cell lines as a potential chemotherapeutic agent.

MATERIALS AND METHODS

Cell Culture

The B164A5 murine melanoma cell line was purchased from the European Collection of Authenticated Cell Culture (ECACC). The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM). This culture media was enriched with 10 % fetal bovine serum, 1 % PenStrep, glucose and L-glutamine. The cells were incubated in a humidified atmosphere at 37 ºC in 5 % CO_2. Sub-culture was done when the cell confluency had reached 80 %.

MTT Assay

The cytotoxicity of QO_2 was evaluated through the 3 - (4,5 - dimethylthiazol - 2 - yl) - 2,5 - diphenyl tetrazolium bromide (MTT) assay by referring to Mosmann (1983) method with slight modifications. About $5x10^4$ cells/mL was seeded on 96-wells plate and incubated for 24 hours at 37 ºC in 5 % CO_2. On the second day, media was discarded and replaced with 100 µL PBS and the cells were subjected for UVB exposure at 30 mj/cm^2 for 36.4 seconds. After that, the PBS was discarded and followed with cell treatment. For positive control, the cells were treated with menadione (0.0625, 0.125, 0.25, 0.5 and 1 mM) where the range of concentrations was adapted from Basri et al (2015). For the treatment group, as the cytotoxicity of QO_2 never been done by anyone before this, hence, we were tried out with the range of concentrations (50, 40, 20, 10 and 5 %, v/v) where the v/v maximum is 50/50. Untreated cells were used as negative control. Another 24 hours incubation at 37 ºC in 5 % CO_2 was followed. On the third day, 20 µL MTT solution (5 mg/mL) was added into each well straight away and the plates were incubated for 4 hours at 37 ºC in 5 % CO_2. Then, 170 µL mixture from each well was discarded and 170 µL dimethyl sulfoxide (DMSO) was added on and further with 15 minutes incubation. Lastly, the plate was shaken for 5 minutes and absorbance reading was taken at 570 nm. Three replicates were done. The percentage of cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{\text{Mean OD of treated cell}}{\text{Mean OD of negative control}} \text{ X100}$$

Statistical analysis

The SPSS v25 software was used for reporting the results. Each data was presented as the mean ± SEM of triplicates from 6 different experiments (n=6). Independent T-test was used for mean comparisons. p value <0.05 was considered as statistically significant.

RESULTS

The cytotoxicity of menadione and QO_2 was evaluated by the MTT assay. Figure 1 shows the cytotoxicity of menadione as a positive control in our study. Menadione exhibited potent cytotoxicity against the treated cells with IC_{50} of 0.03 ± 0.002 mM. Each tested concentration of menadione was

also statistically significant ($p<0.05$) as compared to the negative control.

As for QO_2, two IC_{50} were obtained from the graph shown in Figure 2. The IC_{50} were 2.5 ± 0.08 and 6.5 ± 0.15 % (v/v). There was a reduction in the cell viability (23.02 %) at the beginning of the growth which at the concentration of 5 % (v/v) and then increased to 128.86 % at 10 % (v/v) concentration. Interestingly, at the three consecutive concentrations (10, 20 and 40 %, v/v), the cell viability percentage were found to be exceeding 100 % and the QO_2 at 20 % (v/v) had the highest cell viability which was 132.00 %. Cells at that range of concentrations were actively proliferating until a great reduction of cell viability at 50 % (v/v). Cell viability at QO_2 concentrations of 5 and 50 % (v/v) were significantly ($p<0.05$) lower than the negative control but found to be the opposite at QO_2 10 and 20 % (v/v), which were significantly ($p<0.05$) higher. As for the untreated group, it was regarded as negative control and the results showed no cytotoxicity as no compound was used.

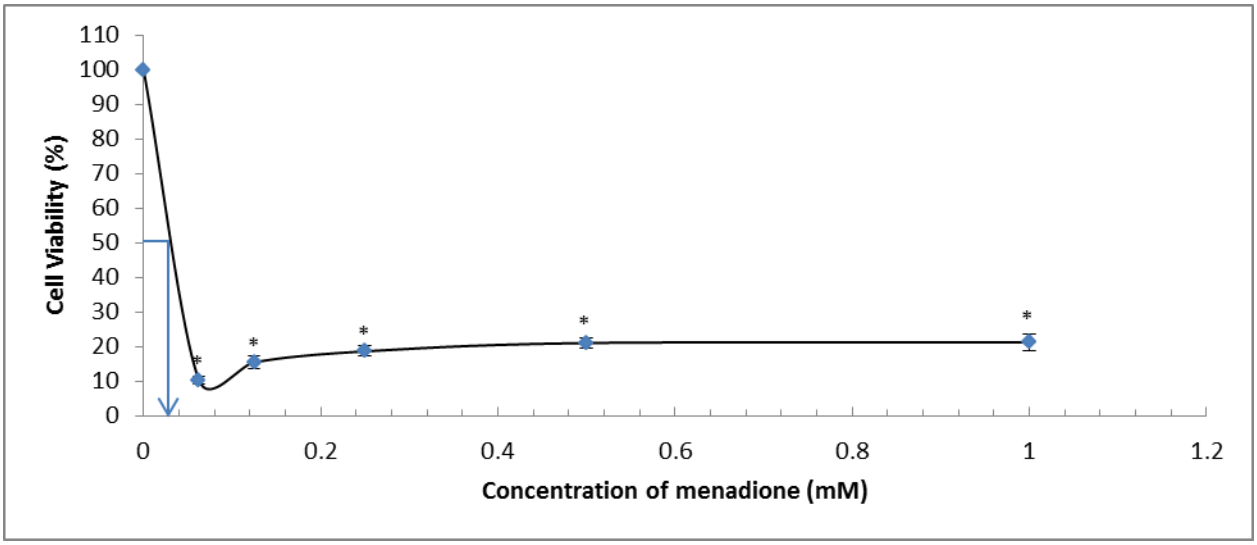

FIGURE 1. Cytotoxicity of menadione against UVB induced B164A5 melanoma cell lines.
Each point represents the mean ± SEM of triplicates from 6 different experiments (n=6).
*Shows statistically significant compared to negative control, $p<0.05$.

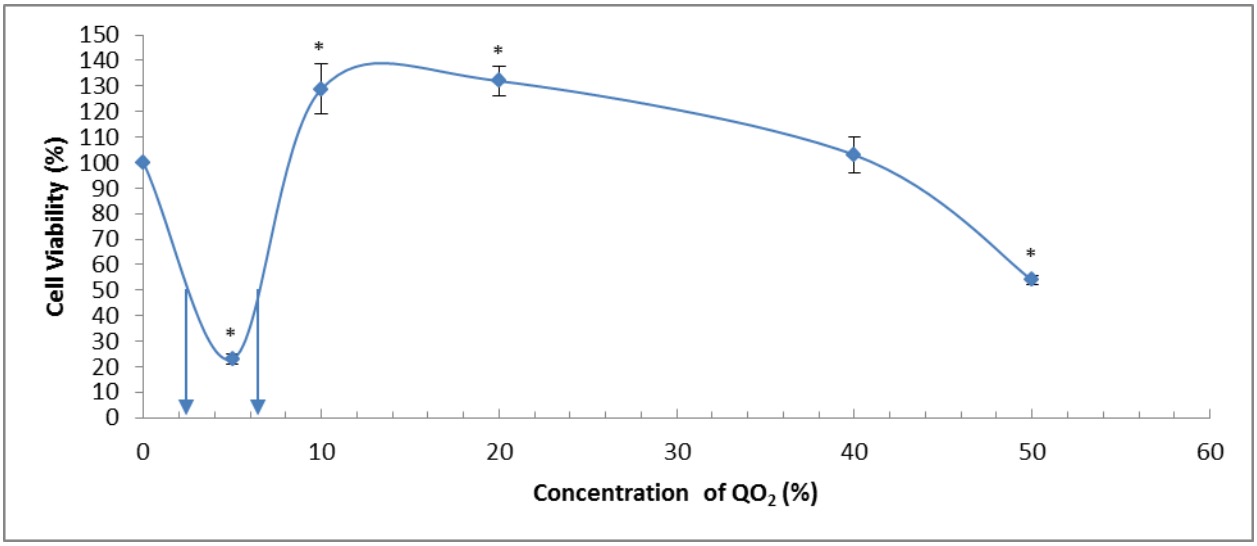

FIGURE 2. Cytotoxicity of QO_2 against UVB induced B164A5 melanoma cell lines.
Each point represents the mean ± SEM of triplicates from 6 different experiments (n=6).
*Shows statistically significant compared to negative control, $p<0.05$.

DISCUSSION

QO_2 is a local water product that has been manufactured as a sanitizer and deodorizer too. By using the special quantum entanglement technology, the oxygen molecules in the reverse osmosis water are impregnated with boosted energy according to the company's official website.

In our study, QO_2 was evaluated for its cytotoxicity by MTT assay against the UVB induced B164A5 melanoma cell lines. 30 mj/cm^2 dose was used to irradiate the cells as described by Satou et al (2019). In order to achieve this 30 mj/cm^2 dose, the cells were needed to be exposed for 36.4 seconds. Based on the cytotoxicity results, it was found that the menadione which acted as a positive control in the study showed cytotoxicity with IC_{50} of 0.03 ± 0.002 mM. Menadione was known as a potent cytotoxicity agent and killed the cells by producing oxidative stress through redox cycle (Chung et al. 1997). Our finding was in line with the other study by Osada et al. (2018) where the IC_{50} was 0.0421 mM against the pancreas cancer. Another IC_{50} on Hacat cells with menadione by Suresh et al. (2013) also showed similar value (0.0745 mM).

Our results also show that QO_2 had cytotoxicity against the treated cells with two IC_{50} which were 2.5 ± 0.08 and 6.5 ± 0.15 % (v/v). In contrast, the following three consecutive concentrations of QO_2 at 10, 20 and 40 % (v/v) exhibited high cell viability which also exceeded more than 100 % viability. Our study postulated that QO_2 demonstrated biphasic effects on the treated cells. This biphasic effects also were demonstrated by Guo et al (2004) but low dose of daidzein (phytoestrogen found in soybean) caused cell proliferation while high dose caused cytotoxicity against human colon cancer cells.

QO_2 possibly acted as a pro-oxidant at the lower range of concentrations but exhibited antioxidant activity at higher concentrations. This trend was similar to Philis-Tsimikas et al (1995) where they found that aminoguanidine was pro-oxidant and antioxidant at low and high concentrations respectively towards low density lipoprotein. The higher QO_2 concentrations then provided the antioxidant activity which could scavenge the free radicals that were produced during UVB exposure and protected the cells from being damaged and led to further proliferation of the cells. In addition, QO_2 can also be suggested to induce apoptosis at low concentrations but not at higher concentrations. This can be supported by Slipicevic et al (2013) where they demonstrated that ansiomcycin (a protein translation inhibitor) induce apoptosis at the low dose by sensitizing the melanoma cells to tumor necrosis factor related apoptosis-inducing signal (TRAIL).

CONCLUSION

In conclusion, QO_2 showed cytotoxic effects at lower concentrations but not at the higher concentrations. The biphasic effects proposed that QO_2 could act as a pro-oxidant at lower concentrations and inducing apoptosis but acted as an antioxidant at higher concentrations. Further investigations to determine the mechanisms of cytotoxicity and cell death of QO_2 on UV irradiated B164A5 melanoma cell lines are still much needed.

REFERENCES

Basri, D. F., Shabry, A. S. M. & Meng, C. K. 2015. Leaves Extract from *Canarium odontophyllum* Miq. (Dabai) Exhibits Cytotoxic Activity against Human Colorectal Cancer Cell HCT 116. *Natural Products Chemistry & Research* 3(2).

Berwick, M., Erdei, E. & Hay, J. 2009. Melanoma Epidemiology and Public Health. *Dermatol Clin* 27(2): 205-214.

Chung, J. H., Seo, D. C., Chung, S. H., Lee, J. Y. & Seung, S. A. 1997. Metabolism and Cytotoxicity of Menadione and Its Metabolite in Rat Platelets. *Toxicology and Applied Pharmacology* 142(2): 378-385.

De Jager, T. L., Cockrell, A. E. & Du Plessis, S. S. 2017. Ultraviolet Light Induced Generation of Reactive Oxygen Species. *Adv Exp Med Biol* 15-23.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A. & Scott, T. 2013. UV Radiation And The Skin. *International Journal of Molecular Sciences 14*(6): 12222-12248.

Gandini, S., Autier, P. & Boniol, M. 2011. Reviews on Sun Exposure and Artificial Light and Melanoma. *Prog Biophys Mol Biol* 107(3): 362-366.

Guo, J. M., Xiao, B. X., Liu, D. H., Grant, M., Zhang, S., Lai, Y. F., Guo, Y.B. & Liu, Q. 2004. Biphasic Effect of Daidzein on Cell Growth of Human Colon Cancer Cells. *Food and Chemical Toxicology*, 42(10): 1641-1646.

International Agency for Research on Cancer. https://www.wcrf.org/dietandcancer/cancer-trends/skin-cancer-statistics

Mosmann, T. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal of immunological methods* 65(1-2): 55-63.

Osada, S., Tomita, H., Tanaka, Y., Tokuyama, Y., Tanaka, H., Sakashita, F. &Takahashi, T. 2008. The Utility of Vitamin K3 (Menadione) Against Pancreatic Cancer. *Anticancer Research* 28(1A): 45-50.

Penta, D., Somashekar, B. S. & Meeran, S. M. 2018. Epigenetics of Skin Cancer: Interventions by Selected Bioactive Phytochemicals. *Photodermatol Photoimmunol Photomed* 34(1): 42-49.

Philis-Tsimikas, A., Parthasarathy, S., Picard, S., Palinski, W. & Witztum, J. L. 1995. Aminoguanidine Has Both Pro-Oxidant and Antioxidant Activity Toward LDL. *Arteriosclerosis, Thrombosis, and Vascular Biology* 15(3): 367-376. QO_2 Official Website. https://qo2.co/

Satou, G., Maji, D., Isamoto, T., Oike, Y. & Endo, M. 2019. UV-B-Activated B16 Melanoma Cells or Hacat Keratinocytes Accelerate Signaling Pathways Associated with Melanogenesis via ANGPTL 2 Induction, An Activity Antagonized by Chrysanthemum Extract. *Experimental Dermatology* 28(2): 152-160.

Slipicevic, A., Øy, G. F., Rosnes, A. K. R., Stakkestad, Ø., Emilsen, E., Engesæter, B., Maelandsmo. G. M. & Flørenes, V. A. 2013. Low-Dose Anisomycin Sensitizes Melanoma Cells to TRAIL Induced Apoptosis. *Cancer Biology & Therapy* 14(2): 146-154.

Strickland, L. R., Pal, H. C., Elmets, C. A. & Afaq, F. 2015. Targeting Drivers of Melanoma with Synthetic Small Molecules and Phytochemicals. *Cancer Lett* 359(1): 20-35.

Suresh, S., Raghu, D. & Karunagaran, D. 2013. Menadione (Vitamin K3) Induces Apoptosis of Human Oral Cancer Cells And Reduces Their Metastatic Potential By Modulating The Expression Of Epithelial To Mesenchymal Transition Markers And Inhibiting Migration. *Asian Pac J Cancer Prev* 14(9): 5461-5.

Wang, S., Shen, P., Zhou, J. & Lu, Y. 2017. Diet Phytochemicals and Cutaneous Carcinoma Chemoprevention: A Review. *Pharmacol Res* 119: 327-346.

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