Impact Of Photodynamic Treatments On Cultured Malignant Melanoma Cell Death And The Associated Biomolecular Changes As Determined By Synchrotron Infra Red Microspectroscopy

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ABSTRACT

Malignant melanoma is a very serious skin cancer that, if not discovered early and treated by surgery can become fatal. Photodynamic treatment (PDT) involves the use of a photosensitive dye that is not toxic by itself and is taken up by normal and tumor cells. However, it is released from normal cells in a short time while it is kept inside tumor cells for a longer time. A laser light of wavelength that is maximally absorbed by the dye is used to illuminate the tumor cells containing the dye, and a reaction takes place resulting in the liberation of "singlet oxygen", a very active form of oxygen that damages important biomolecules and results eventually in cell death. The photosensitive dye, indocyanine green, and a Ti - Sapphire laser (780 nm wavelength and power density of 55 mWatt/ cm^2) were used. Human malignant melanoma cells were obtained locally and cultured and fed with standard medium . Photodynamic treatments were carried out with the dye at a concentration of 150 µM and at various laser exposure times (0 – 60 minutes). The vital stain trypan blue was used to assess the percentage of cell death as compared with the controls. Results showed that the percentage of cell death increased slowly with the strength of the PDT until it reached 75% death of the exposed cells at a laser exposure time of 60 minutes. Synchrotron infrared imaging was carried out on control and experimental cultures at the National Synchrotron Light Source (BNL) using Fourier Transform Infra Red (FTIR) microspectroscopy to reveal the infrared absorption characteristics of protein, lipids and nucleic acids biomolecules. It was found that increasing the strength of the PDT had an increasingly damaging effect on the cellular biomolecules, where the largest effect occurred in the lipid , protein and nucleic acid components of the cell. Future experiments are planned involving changes in the strength of the PDT treatment to eventually reach 100% melanoma cell death.

1. INTRODUCTION

Melanoma cells are malignant cells that develop from melanocytes , which are cells in the skin that produce melanine pigment . As with most cancers , once melanoma cells develop they can quickly metastasize . Typical treatment involves removal of a melanoma tumor with surgery, before progression of the disease and its infiltration into deeper skin layers .

Another treatment that is currently being explored is photodynamic treatment (PDT)(1-4) , where a photosensitive dye is taken up by normal and tumor cells but leaves normal cells after a short time . The cells are then exposed to laser light of wave length that is maximally absorbed by the used dye. This results in the liberation of a very active form of oxygen (singlet oxygen) which damages the cell biomolecules and results in cell death . Indocyanine green is a common photosensitive dye (5-8)

Since photodynamic treatment has not been used routinely in the case of malignant melanoma for its treatment , a series of experiments were planned using cell cultures of melanoma cells :

(a) To arrive at an optimum dye concentration and laser exposure dose that causes cultured melanoma cell death .

(b) To find out , using Fourier Transform Infra Red (FTIR) Spectromicroscopy the changes in the I.R. spectra of cell biomolecules with the photodynamic treatment using I.R. from a synchrotron, were investigated (9-13)

2. MATERIALS AND METHODS

Melanoma cell cultures

- HBT72 Human malignant melanoma cells were obtained locally from cell banks and were fed with the appropriate medium. The cultures, both controls and experimental cultures, were maintained in 32 mm plastic culture dishes and in which were placed specially treated low e-slides for some of the cells to attach to it in preparation for photodynamic treatments. After 3 days, the culture medium used was replaced with indocyanine green dye (150µM). After 4 hours of dye exposure the solution in the dishes was replaced with media.
- At the dye concentration used, no significant toxicity (cell death) compared to cell cultures that had no dye was observed.

- Similarly, no toxicity was caused by laser exposure alone and no significant rise in the medium temperature by the highest laser exposures was observed.
- Several dishes underwent different laser exposures (15,30,45,60) minutes at 55mW/cm**2** dose.
- After exposure the medium in each dish was once again replaced and the dishes were reincubated for 24 hours.

Cell counting

- 24 hours after laser exposure, the media were aspirated off the dishes ,.Low e-slides were removed and rinsed with 70% ethyl alcohol to fix the cells. These were used for infrared data collection to find out changes ,if any ,in the cell biomolecular spectra with the PDT treatment .
- The vital stain trypan blue was used to assess the percentage of cell death due to the various photodynamic treatments (PDT) as compared with the controls..
- The cells in the pertri dish were trypsinized and then treated with trypan blue in a 1: 200 µL dilution
- Cell counts were then performed on a 10 µL aliquot in a hemocytometer .Cells that stained blue were dead and cells not stained were alive

3. RESULTS

The two main experimental goals were:

A) The biomolecular changes resulting from the different levels of photodynamic treatments were investigated by comparing their I.R. cellular biomolecular spectra after with that of the biomolecular spectra for the biomlecules : lipids , proteins and nucleic acids .(Fig .1,2,3) after PDT.

B) Percent cell killing observed at the different photodynamic treatments used are presented in a bar diagram (Fig.4).

The bar diagram shows that , at a constant dye concentration , increasing the laser exposures result in an increase in % cell death to about 75% , (using trypan blue stain which stains cells that have lost cell membrane integrity) at a laser exposure of 60 minutes (Fig 4) . In addition the absorbance (ln one over R where R is the I.R. reflections from the Low-e slide on which the cells lie) spectra for the different photodynamic treatments reflect decreased absorbance , i.e damage for the different cellular biomolecules with increasing the duration of the photodynamic exposures , (Fig 3) .

4. DISCUSSION

Using trypan blue vital stain it can be observed from the results obtained that the photodynamic treatment (PDT) has an increasingly damaging effect on cultured melanoma cells with increasing the level of PDT. This is shown in both the bar diagram and the I.R. biomolecular spectra of these cells compared to the controls. Cell death increased with PDT increase. Biomolecular damage ,especially for lipids, leads eventually to cell membrane damage (necrosis) resulting in cell death.

However it should be emphasized that , cell culture , is only a model of the real situation of a melanoma skin cancer. Hence there is definitely a need to test the PDT on an in vivo animal model. The photosensitive dye , for example indocyanine green , can be added to a cream or ointment and applied to the skin melanoma patches and then exposed to the appropriate laser dose. Hopefully this in vivo test will succeed and PDT for melanoma will then be on the way to become another option beside or even replace surgical treatment. It should also be emphasized here that the laser power used in this investigation , namely 55 m watt/cm2 ,is much smaller than the conventional power (about 500 mwatt/cm2) used in conventional dermatology. This means that much less exposure time will be needed in actual treatments.

5. CONCLUSIONS

Photodynamic treatment (PDT) of cultured human melanoma cells resulted, according to the strength of the treatment , in cell death that increased with PDT. Cell death was caused by the formation of a very toxic form of oxygen , namely singlet oxygen which damages cell biomolecules such as proteins , lipids , and nucleic acids resulting in cell death revealed by the vital stain trypan blue . These results when confirmed by an animal model paves the way for a non surgical treatment for malignant melanoma.

6. REFERENCES

[1] BROWN, S., (1999), Another Key Milestone for Photodynamic therapy *Photodynamic News*, 2, 1.

[2] DOUGHERTY, T., GAMER C., HENDERSON, B., JOIR, G., KESSEL, D., KORBELIK, M., MOAN, J. PENG, Q., (1998) PhotodynaInic Therapy, *J., Nat. Cancer Instit.*, Vol. 90. pp. 889-905.

[3] SHAW, S.,(2000) Shining light on Cancer: Promise and problems of Photo sensitizers, *Science Spectra*, Issue 21.

[4] Kessel, D.& Luo ,Y., (1998) Mitochondrial photodamage and PDT-induced apoptosis *J. Photochem Photobiol B* ., 42,89-95.

[5] KRYSTYNA URBANSKA, BOZENA ROMANOWSKA – DIXON, ZENON MATUSZAK, JANUSZ OSZAJCA, PATRYCIA NOWAK – SLIWINSKA, GRAZYNA STOCHE, (2002) Indocyanine green as a prospective sensitizer for photodynamic therapy of melanomas, *Acta Biochimica Polonica*, Vol. 49, No 2.

[6] KYUNG - SUN CBO , EUNJOO H .LEE ,JUN – SUB CBOI, CBOUN - KI JOO, (1999), Reactive Oxygen Species – Induced Apoptosis and Necrosis in Bovine Corneal Endothelial Cells - *Investigative Ophthalmology & Visual Science* , April, Vol . 40 , no . 5.

[7] HIN - FAI YAM, ALVIN KWAN - HO KWOK, KWOK - PING CHAN, TIMOTHY YUK - YOU LAU ,KWAN - YI CBU, DENNIS SBUN - CBIU. Lam, CBI PUI PANG, (2003) Effect of Indocyanine Green and Illumination on Gene Expression in Human Retinal Pigment Epithelial Cells, *Investigative Ophthalmology& Visual Science*, Vol .44. no 1, pp370 – 377.

[8] ELVIRA CRESCENZI, LINDA VARRIALE, MARIANGELA lOVINO, ANGELA CHIAVIELLO, BIANCA MARIA VENEZIANI, GIUSEPPE PALUMBO, (2004), Photodynamic therapy with indocyanine green complements and enhances low - dose cisplatin cytotoxicity in MCF – 7 breast cancer cells , *Molecular Cancer Therapeutics* ,: 3(5) , pp . 537 – 544.

[9] HOI-YING N. HOLMAN, REGINE GOTH - GOLDSTEIN, MICHAEL C. MARTIN, MARION L. RUSSELL, WYNE R. MCKINNEY,(2000), Low Dose Responses to 2,3,7,8 – Tetrachlorodibenzo – p – dioxin in Single Living Human cells measured by Synchrotron Infrared Spectromicroscopy. *Environ. Sci. Technol* ., 34, 2513-2517

[10] HOI - YING N. HOLMAN, MICHAEL C. MARTIN, ELEANOR A. BLAKELY, KATHY BJORNSTAD, WAYNE R. MCKINNEY, (2000), IR Spectromicroscopic Characteristics of Cell Cycle and Cell Death Probed by Synchrotron Radiation Based Fourier Transform IR Spectromicroscopy, *Biopolymers (Biospectroscopy)*, vol. 57, 329-335.

[11] MICHAEL C. MARTIN, WAYNE R. MCKINNEY, (2001), The First Infrared Beam lines at the Advanced light Source: Spectromicroscopy and Fast Timing, *Ferroeletrics*, vol. 249 (1-2), pp.1-10.

[12] MICHAEL C. MARTIN, NELLY M. TSVETKOVA , JOHN H. CROWE, WAYNE R. MCKINNEY, (2001), Negligible Sample Heating from Synchrotron Infrared Beam, *Applied Spectroscopy*, vol. 55, No.2, pp. 111 - 113 .

[13] HOI - YING N. HOLMAN, KATHLEEN A. BJORNSTAD . MORGAN P. MCNAMARA, MICHAEL C. MARTIN, WAYNE R. MCKINNEY, ELEANOR A . BLAKELY,(2002), Synchrotron infra red spectromicroscopy as a novel bioanalytical microprobe for individual living cells : cytotoxicity considerations, *Journal of Biomedical Optics* , vol . 7, No 3 , pp.417 – 424