PHOTOBIOMODULATION OF QUERCETIN ANTIPROLIFERATIVE EFFECTS SEEN IN HUMAN ACUTE T LEUKEMIC JURKAT CELLS

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Abstract. In this report we investigated the effects of low power 680 nm far-red and 830 nm near-infrared laser irradiation on quercetin-induced cell growth inhibition and apoptosis induction. High micromolar concentrations (100μM) of quercetin decreased cell viability and prevented the proliferation of human T leukemic lymphoblasts (Jurkat) in a time-dependent manner. Flow cytometry data attested cell cycle arrest in the S phase and apoptosis induction. Low dose (~1 μJ/cell) laser irradiation significantly modulated the quercetin effects seen in human leukemic cells. Near-infrared laser light partially reversed apoptosis induction and promoted cell cycle progression in the G2 phase, in a dose-dependent manner, while far-red laser light potentiated quercetin-toxicity.

Key words: AlGaInP/GaAs laser, quercetin-induced stress, viability, proliferation rate, cell cycle progression, apoptosis.

INTRODUCTION

Flavonoids, a large family of polyphenolic compounds synthesized by plants, were shown to possess antioxidant, anti-inflammatory, anti-allergic, cancer chemo-preventive, anti-cancer and anti-tumor activities of significant potency and low systemic toxicity [3, 6, 12, 13, 15–19, 21, 22, 25, 27, 31–33]. Compelling data suggest that dietary flavonoids may contribute to cancer prevention, and many of them were considered for clinical evaluation of their efficacy and development of novel complementary/alternative therapeutic strategies. Nonetheless, the detailed molecular and cellular mechanisms involved remain elusive and little information is available regarding possible synergistic or antagonistic biochemical interactions among these compounds and among these compounds and other therapeutic regimes [1, 17, 29, 31].

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A similar situation as for low level laser therapy (LLLT) already part of physiotherapy in most countries is successfully used whenever the goal is promotion of wound healing, reduction of inflammation and/or pain relief [24, 26, 28]. In spite of the growing number of well designed scientific investigations [7–9, 23, 30], many molecular mechanisms underlying short term and long term effects of soft laser irradiation should yet be thoroughly elucidated in order to allow improvement of the effective but so far empirical treatment protocols.

Among plant flavonoids, quercetin (3,3',4',5,7-pentahydroxyflavone) has also been repeatedly reported to induce cell growth inhibition and apoptosis in a variety of cancer cells [6, 12, 13, 15, 17–19, 25, 27, 32, 33]. On the other hand, there is growing evidence concerning low level red and infrared laser irradiation beneficial effects promoting various cells proliferation [2, 5, 11, 23] and restoring function of intoxicated neural cells [4, 7, 14, 20, 30], and we also observed metabolically modulated effects of the 680 and 830 nm laser light on blood cells viability and cell proliferation [10]. This study was designed to investigate modulation by these far-red and near-infrared lasers light of quercetin effects occurring in the human T leukemia lymphoblasts (Jurkat). We assessed cell viability, proliferation rate, and cell cycle progression. Data obtained attest apoptosis induction, inhibition of proliferation and cell cycle arrest by quercetin at a dose of 100 μM in 18–54 h, and significant influence of the low level (~1 μJ/cell) laser irradiation, more evident in treated than in control Jurkat cells. Near-infrared light seemed to reverse quercetin effects, while far-red irradiation appeared to promote quercetin toxicity.

MATERIALS AND METHODS

Chemicals, supplements and staining kits: quercetin, sterile DMSO, Trypan Blue, and standard RPMI 1640 culture medium and were purchased from SIGMA CHEMICAL Co., Dulbecco's Phosphate buffered Saline (PBS) 10× from BIOCHROME, while supplements (FCS, fetal calf serum, and antibiotics/glutamine) from GIBCO/INVITROGEN. The propidium iodide (PI) / RNAse staining buffer was from BD PHARMINGEN, while all other chemicals were the best research grade available.

Stock solutions and modified culture media: quercetin was dissolved in DMSO, realizing stock solutions of 100 mM. Modified culture media were prepared from standard RPMI medium: complete medium supplemented with 10% FCS (M), serum deprived medium (M0 – M without FCS), quercetin-containing medium (M100Q – M containing 100 μM quercetin).

Cell culture: the human acute T leukemic Jurkat cell line was maintained in a 5% CO_2 humidified (80%) atmosphere at 37 °C, in standard RPMI 1640 medium (SIGMA R6504) supplemented with 2 g/l sodium bicarbonate, 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and passaged at every second day.

Serum starvation: cells were resuspended in M0 medium and incubated in a 5% CO_2 humidified (80%) atmosphere at 37 °C for the indicated time periods (12–24 h).

Quercetin treatment: cells were incubated in a 5% CO_2 humidified (80%) atmosphere at 37 °C for various time periods in 60–100 μM quercetin-containing media. Quercetin stock solutions of 100 mM in DMSO were used, so that DMSO in culture media never exceeded the concentration known not to affect the cell proliferation 0.1% (v/v).

The irradiation sources were AlGaInP/GaAs based semiconductor lasers used in the medical practice, PHILIPS CQL806D and SONY SLD202-D3, with emission wavelengths and nominal powers of 680 nm at 25 mW, and of 830 nm at 50 mW, respectively.

Sample irradiation regimes and laser irradiation doses: sample irradiation was performed in the laminar flow, with sources placed at a 10 cm height from the upper surface of cell suspensions, in 2 consecutive positions as to cover with the expanded laser speckle the whole surface of the suspension-containing Petri dishes rotating at a speed of 0.5 s^{-1}. Duration of treatment varied between 0–600 s, giving at the average incident power densities in the expander–increased speckle area of approximately 45 $mW \times cm^{-2}$ and 100 $mW \times cm^{-2}$ for the red and infrared laser beam, respectively, single incident doses equivalent with $(1 \div 5) \times 10^{12}$ photons/cell or ~ (0.2 ÷1.5) μJ/cell. Irradiation regimes of once or twice per day, or every second day with these single doses gave total irradiation doses of ~ (1–15) μJ/cell.

Cell viability and cell growth assay: after 16 h of serum starvation, cell suspensions at a density of 0.92×10^6 cells/ml in various media, distributed in 3.5 cm diameter Petri dishes, were cultured for 60 h. At indicated time points cells in aliquots of 50 μl were counted in a Buerker-Tuerk hemocytometer. Cell viability was tested by the Trypan Blue exclusion method.

Cell fixation: at indicated time points, 10^6 cells were washed with PBS, and fixed at least 2 h in 70% ethanol. Fixed samples were washed, stained and measured or kept 4–6 weeks at –20 ^oC, until the day when they were stained with the purpose of cell cycle analysis, and analysed.

Labeling of cells for cell cycle analysis: propidium iodide (PI) staining was performed using a PI/RNAse staining buffer (PHARMINGEN 550825) according to manufacturer protocol. Briefly, at indicated time points, 10^6 cells – fresh samples or previously fixed, kept at –20 ºC – were washed with PBS, and incubated with the staining buffer for 30 minutes at 37 ºC in the dark.

Cell cycle analysis: cells were analyzed for DNA content by a Becton Dickinson FACS Calibur flow cytometer, CellQuestas dedicated data acquisition software. The freeware WinMDI 2.8 was used for raw data analysis, and the histogram deconvolution software Cylchred for cell cycle distribution quantization.

Data analysis: viabilities and cell numbers were obtained as means calculated from at least 3 independent measurements (S.D. ≤ 12%). Relative changes occurred in these parameters under the influence of quercetin treatment and/or of laser irradiation were calculated as ratios of differences between averages calculated from values measured on 3 independent treated/irradiated samples and the control average value calculated from values measured on 3 independent untreated samples and this control average. Unpaired analysis of data series obtained by measurements made on cells various time periods after their transfer in quercetin-containing media, irradiated and/or not irradiated, was performed by Student's t-test (two-tailed). P-values less than 0.05 were regarded as indicating statistical significance. Percentages of cell populations in various cell cycle phases were calculated as averages of values given by the WinMDI software with marker defined histogram regions, and of values given by the deconvolution software Cylchred, from measurements made on at least 2000 single cells.

RESULTS

Assays were performed to determine Jurkat cells viability and proliferation, and changes occurring in these parameters in quercetin-treated and/or irradiated samples. Counting live and dead cells in Trypan Blue stained samples in a hemocytometer, following 18–54 h exposure to a dose of 60–100 μM quercetin, the flavonoid-induced cytotoxicity and cell growth inhibition was evident, as well as the sensitive modulation by soft laser irradiation of the observed effects. Figure 1 illustrates the viability decrease apparent in cells exposed to 100 μM quercetin for 47 h, and the statistically significant modulation of this decrease under the influence of a single irradiation dose of ~1 μJ/cell of 830 nm near-infrared and 680 nm far-red laser light, respectively. Figure 2 illustrates the changes occurring in the live cells number following a 40 h exposure of Jurkat lymphoblasts to a 100 μM quercetin dose, as well as the modulation of proliferation or death rate, noticeable in control and quercetin-treated samples, under the influence of a single irradiation dose of ~1 μJ/cell of 830 nm near infrared and 680 nm far-red laser light, respectively.

DNA content analyses by flow cytometry indicated significant alterations in cell cycle kinetics of Jurkat cells under the influence of quercetin (at a dose of 100 μM for 24 and 53 h). A typical cell cycle distribution in Jurkat cells and the deconvolution of DNA-content frequency histograms are illustrated in Fig. 3, while distorted DNA content histograms registered in quercetin-treated samples, indicating altered cell cycle progression – blockade of cell cycle in the S phase and increase in the apoptotic cell population – are shown in Fig. 4. Significant modulation of quercetin-effects by red and infrared laser irradiation is evident once more.

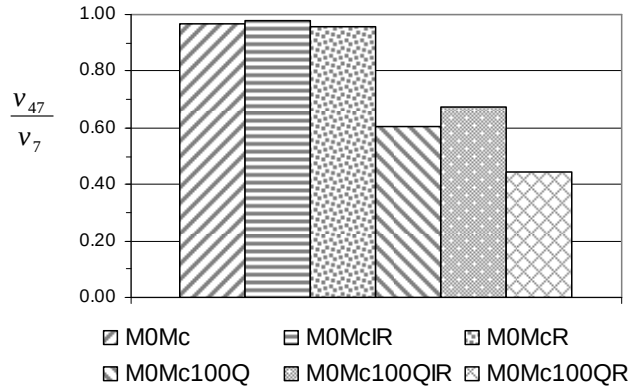

Fig. 1. Quercetin and laser irradiation effects on relative viability of Jurkat cells 47 h after their resuspension in various media. Jurkat cells resuspended after 16 h of serum starvation (M0) in standard RPMI medium Mc (M0Mc) or in medium containing 100 μM Quercetin, some of them being irradiated with red (R, 680 nm, 1.05 μJ/cell) and infrared (IR, 830 nm, 0.86 μJ/cell) respectively, 8 h after their resuspension v_{47} and v_7 are cell viabilities determined by the trypan exclusion method, counting trypan excluding and trypan not excluding cells in a Buerker-Tuerk hemacytometer, using a Zeiss Axiovert 25CFL microscope.

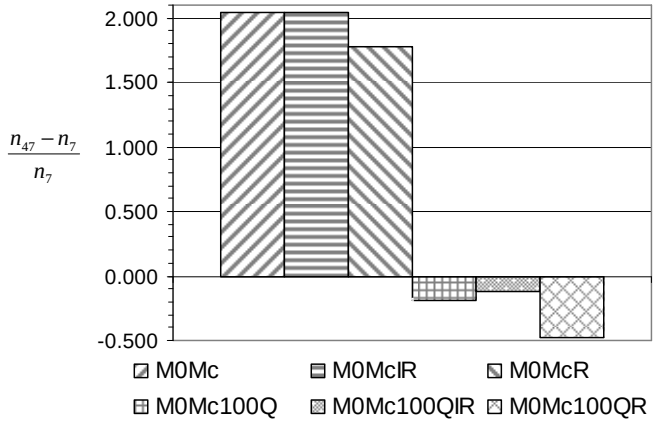

Fig. 2. Quercetin and laser irradiation effects on relative cell concentration increase in 40 h of Jurkat cells, measured 47 h after their resuspension in various media. Jurkat cells resuspended after 16 h of serum starvation (M0) in standard RPMI medium Mc (M0Mc) or in medium containing 100 μM Quercetin (Mc100Q), some of them being irradiated with red (R, 680 nm, 1.05 μJ/cell) and infrared (IR, 830 nm, 0.86 μJ/cell) laser light respectively, 8 h after their resuspension n_{47} and n_7 are the cell numbers in unit volume, counted in a Buerker-Tuerk hemacytometer using a Zeiss Axiovert 25CFL microscope, 47 and 7 h after cells resuspension in media, respectively.

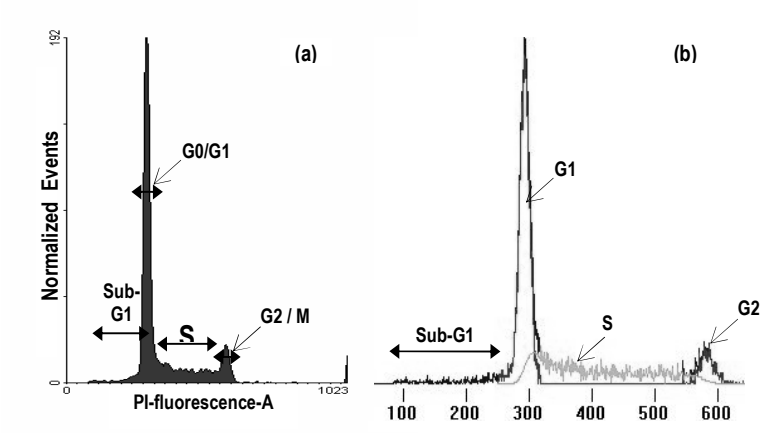

Fig. 3. Typical cell cycle distribution in Jurkat cells stained with Propidium Iodide (PI) inferred from DNA content distribution presumed as being proportional with PI-fluorescence intensity (a), and deconvolution of DNA-content-frequency histogram to estimate the percentage of cells in major phases of the cell cycle and frequency of apoptotic cells with fractional DNA content (b). Cells fixed with 70% EtOH and stained using a PI/RNAse kit (Pharmingen 550825). PI-fluorescence intensity proportional with cellular DNA content, measured by flow cytometry using a BD Facs Calibur instrument. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. The sub-G1 population is considered as being constituted by apoptotic cells. (a) WinMDI histogram with markers; (b) main cell cycle phases revealed by the DNA-content-frequency histogram deconvolution program Cylchred.

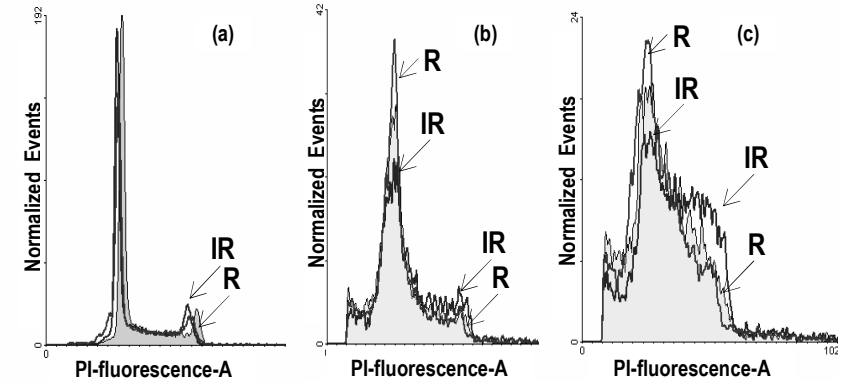

Fig. 4. Quercetin and laser irradiation effects on cell cycle distribution in Jurkat cells stained with Propidium Iodide. Cells fixed with 70% EtOH and stained using a PI/RNAse kit (Pharmingen 550825). PI-fluorescence intensity proportional with cellular DNA content, measured by flow cytometry using a BD Facs Calibur instrument. WinMDI DNA-content-frequency histograms of data registered on samples fixed 24 h (a and b) and 53 h (c) after serum starved (16 h) cells resuspension in standard RPMI medium (a) and in medium containing 100 µM quercetin (b and c) respectively. Control cells and cells irradiated with red (R, 680 nm, 1.05 µJ/cell) and infrared (IR, 830 nm, 0.86 µJ/cell) laser light respectively, 8 h after their resuspension (b and c) and 44 h after their resuspension (c).

Figures 5–8 explicitly illustrate quercetin effects on Jurkat cells distribution in cell cycle stages, as well as photobiomodulation of this distribution – less evident in control and more pronounced in quercetin-treated cells.

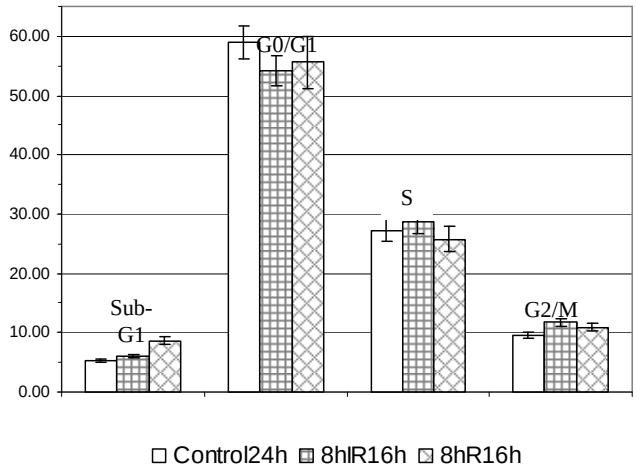

Fig. 5. Photobiomodulation of percentage distribution of Jurkat cells in cell cycle stages, seen 24 h after cells resuspension in complete medium, and 16 h after their irradiation. Jurkat cells resuspended after 16 h of serum starvation (M0) in standard RPMI medium with 10% FCS (Mc), some of them 8 h after their resuspension being irradiated with red (R, 680 nm, 1.05 µJ/cell) and infrared (IR, 830 nm, 0.86 µJ/cell) laser light respectively. The sub-G1 population is constituted of apoptotic cells, while G0/G1, S and G2/M are cell populations in the respective cell cycle stages. Bars represent standard deviations calculated in cell cytometric measurements on 7680, 10330 and 6450 cells respectively. Measurements made on cells fixed in 70% EtOH, and stained using a PI/RNAse Kit (Pharmingen 550825). Data collected on a Becton-Dickinson FACS Calibur flow cytometer. The software used for data analysis and deconvolution of DNA content histograms: WinMDI 2.8 and Cylchred.

DISCUSSION AND CONCLUSIONS

At a dose of 100 µM concentration, quercetin induced a significant decrease in Jurkat cells viability. The viability decrease reaching ~40% following a 47 h exposure, seemed to be partially prevented/reversed by a single dose of ~1 µJ/cell of 830 nm near infrared laser irradiation, while the approximately same dose of 680 nm far-red laser irradiation significantly enhanced the quercetin-induced viability decrease. At the same time cell proliferation was impeded by quercetin; near-infrared laser irradiation appeared to offer some protection, while far-red laser light promoted antiproliferative effects of quercetin.

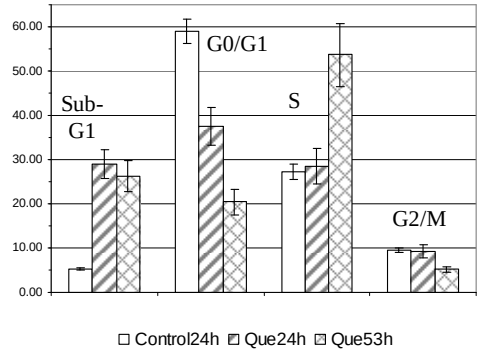

Fig. 6. Quercetin effects on percentage distribution of Jurkat cells in cell cycle stages, seen 24 h and 53 h after cells resuspension in 100 μM Quercetin containing medium. Jurkat cells resuspended after 16 h of serum starvation (M0) in standard RPMI medium with 10% FCS (Mc), containing 100 μM Quercetin. The sub-G1 population is constituted of apoptotic cells, while G0/G1, S and G2/M are cell populations in the respective cell cycle stages. Bars represent standard deviations calculated in cell cytometric measurements on 7680, 4215 and 4935 cells respectively. Measurements made on cells fixed in 70% EtOH, and stained using a PI/RNAse Kit (Pharmingen 550825). Data collected on a Becton-Dickinson FACS Calibur flow cytometer. Software used for data analysis and deconvolution of DNA content histograms: WinMDI 2.8 and Cylchred.

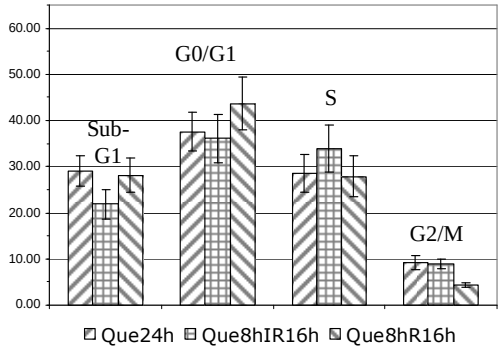

Fig. 7. Photobiomodulation of quercetin effects on percentage distribution of Jurkat cells in cell cycle stages, seen 24 h after their resuspension in 100 μM Quercetin containing medium. Jurkat cells resuspended after 16 h of serum starvation (M0) in standard RPMI medium with 10% FCS, and 100 μM Quercetin (100QMc), some of them being irradiated with red (R, 680 nm, 1.05 μJ/cell) and infrared (IR, 830 nm, 0.86 μJ/cell) respectively, 8 h after their resuspension. The sub-G1 population is constituted of apoptotic cells, while G0/G1, S and G2/M are cell populations in the respective cell cycle stages. Bars represent standard deviations calculated in cell cytometric measurements on 4215, 2445 and 8205 cells respectively. Measurements made on cells fixed in 70% EtOH, and stained using a PI/RNAse Kit (Pharmingen, 550825). Data collected on a Becton-Dickinson FACS Calibur flow cytometer. Software used for data analysis and deconvolution of DNA content histograms: WinMDI 2.8 and Cylchred.

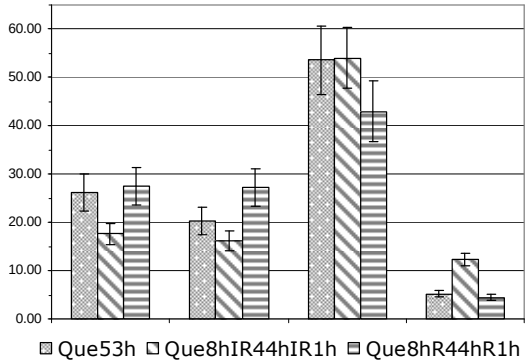

Fig. 8. Photobiomodulation of quercetin effects on percentage distribution of Jurkat cells in cell cycle stages, seen 53 h after their resuspension in 100 μM quercetin containing medium. Jurkat cells resuspended after 16 h of serum starvation (M0) in standard RPMI medium with 10% FCS, and 100 μM Quercetin (100QMc), some of them being twice irradiated with red (R, 680 nm, 1.05 μJ/cell) and infrared (IR, 830 nm, 0.86 μJ/cell) laser light respectively, 8 h and 44 h after their resuspension. The sub-G1 population is constituted of apoptotic cells, while G0/G1, S and G2/M are cell populations in the respective cell cycle stages. Bars represent standard deviations calculated in cell cytometric measurements on 4935, 7800 and 10588 cells, respectively. Measurements made on cells fixed in 70% EtOH, and stained using a PI/RNAse Kit (Pharmingen, 550825). Data collected on a Becton-Dickinson FACS Calibur flow cytometer. Software used for data analysis and deconvolution of DNA content histograms: WinMDI 2.8 and Cylchred.

DNA content analysis by flow cytometry confirmed the 100μM quercetin dose cytotoxicity at exposure periods longer than 18h. Increase of both sub-G1 and S phase cell populations clearly indicate apoptosis induction in Jurkat lymphoid cell line having an S phase entry pathway or checkpoint for programmed cell death. Quercetin induced cytotoxicity increased in time. The 680nm far-red laser irradiation potentiated quercetin effects, further increasing the death-rate, and decreasing cell viability, and the cell population reaching the G2 phase. The 830nm near infrared laser irradiation (doses of 0.9 and 1.8μJ/cell) appeared to offer some protection, slightly increasing quercetin-intoxicated Jurkat cells viability, decreasing apoptosis induction and promoting entry of part of cells in the G2 phase surpassing S phase arrest. The underlying molecular mechanisms are largely unknown and need further investigations.

In summary, our data document photobiomodulation of quercetin cytotoxicity induced through S phase cell cycle arrest and apoptosis induction in human T leukemic lymphoblasts (Jurkat), the 830nm near-infrared laser irradiation being protective partially reversing apoptosis induction and promoting limited cell cycle progression in the G2 phase in a dose-dependent manner, while far-red laser irradiation potentiating quercetin-toxicity.

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REFERENCES

1. ADAMS, L.S., N.P. SEERAM, M.L. HARDY, C. CARPENTER, D. HEBER, Analysis of the interactions of botanical extract combinations against the viability of prostate cancer cell lines, *Evid. Based Complement. Altern. Med.*, 2006, **3**, 117–124.
2. ALMEIDA-LOPES, L. *et al.*, Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence, Lasers Surg. Med., 2001, **29**(2), 179–184.
3. ARAKAKI, N., A. TOYOFUKU, Y. EMOTO, T. NAGAO, Y. KURAMOTO, H. SHIBATA, T. HIGUTI, Induction of G1 cell cycle arrest in human umbilical vein endothelial cells by flavone's inhibition of the extracellular signal regulated kinase cascade, *Biochem. Cell Biol.*, 2004, **82**, 583–588.
4. BYRNES, K.R. *et al*, Cellular invasion following spinal cord lesion and low power laser irradiation, *Lasers Surg. Med.*, 2002, **S14**, 11.
5. CHENG, LEI, TIMON CHENG-YI LIU, JIN-QUAN CHI, YAN LI, HUA JIN, Photobiomodulation on the proliferation and collagen synthesis of normal human skin fibroblast cells. Proceedings of the SPIE, 2006, **6026**, 63–72.
6. CHOI, J.A., J.Y. KIM, J.Y. LEE, C.M. KANG, H.J. KWON, Y.D. YOO, T.W. KIM, Y.S. LEE, S.J. LEE, Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin, *Int. J. Oncol.*, 2001, **19,** 837–844.
7. EELLS, J.T., M.M. HENRY, P. SUMMERFELT, M.T.T. WONG-RILEY, E.V. BUCHMANN, M. KANE, N.T. WHELAN, H.T. WHELAN, Therapeutic photobiomodulation for methanol-induced retinal toxicity, PNAS, 2003, **100**(6), 3439–3444.
8. KARU, T., L.V. PYATIBRAT, G.S. KALENDO, Photobiological modulation of cell attachment via cytochrome c oxidase, *Photochem. Photobiol. Sci.*, 2004, **3**, 211–216.
9. KARU, T, L.V. PYATIBRAT, S.F. KOLYAKOV, N.I. AFANASYEVA, Absorption measurements of a cell monolayer relevant to phototherapy: Reduction of cytochrome c oxidase under near IR radiation, *J. Photochem. Photobiol. B: Biology*, 2005, **8507**, 23–32.
10. KATONA, GY., *et al.*, Metabolic modulation of low power long wavelength laser irradiation effects, seen in human T cells, *European Biophys. J.*, 2005, **34**, 759.
11. KREISLER, M., A.B. CHRISTOFFERS, B. WILLERSHAUSEN, B. D'HOEDT, Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study, *J. Clin. Periodont.*, 2003, **30**(4), 353.
12. KUO, P.C, HUEI-FANG LIU, JUI-I CHAO, Surviving and p53 modulate quercetin-induced cell growth inhibition and apoptosis in human lung carcinoma cells, *J.Biol.Chem.*, 2004, **279**, 55875–55885.
13. LEE, T., O. KIM, Y. KIM, J. LIM, S. KIM, J. PARK, T. KWON, Quercetin arrests G2/M phase and induces caspase-dependent cell death in U937 cells, *Cancer Letters*, 2006, **240**, 234–242.
14. LIANG, H.L., H.T. WHELAN, J.T. EELLS, H. MENG, E. BUCHMANN, A. LERCH-GAGGL, M. WONG-RILEY, Photobiomodulation partially rescues visual cortical neurons from cyanide-induced apoptosis, *Neuroscience*, 2006, **139**, 639–49.
15. LU, J., L.V. PAPP, J. FANG, S. RODRIGUEZ-NIETO, B. ZHIVOTOVSKY, A. HOLMGREN, Inhibition of mammalian thioredoxin reductase by some flavonoids: Implications for myricetin and quercetin anticancer activity, *Cancer Research*, 2006, **66**, 4410–4418.
16. MAYR, G.W., S. WINDHORST, K. HILLEMEIER, Antiproliferative plant and synthetic polyphenolics are specific inhibitors of vertebrate inositol-1,4,5-trisphosphate 3-kinases and inositol polyphosphate multikinase, *J. Biol. Chem.*, 2005, **280**, 13229–13240.

17. MERTENS-TALCOTT, S.U., S.T. TALCOTT, SUSAN S. PERCIVAL, Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells, *J. Nutr.*, 2003, **133,** 2669–2674.
18. MERTENS-TALCOTT, S.U., J.A. BOMSER, C. ROMERO, S.T. TALCOTT, S.S. PERCIVAL, Ellagic acid potentiates the effect of quercetin on p21waf1/cip1, p53, and MAP-kinases without affecting intracellular generation of reactive oxygen species *in vitro*, *J. Nutr.*, 2005, **135**, 609–614.
19. MOUAT, M.F., K. KOLLI, R. ORLANDO, J.L. HARGROVE, A. GRIDER, The effects of quercetin on SW480 human colon carcinoma cells: a proteomic study, *Nutrition Journal*, 2005, **4,** 11–17.
20. ORON, A. *et al*, Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits, *Stroke*, 2006, **37**, 2620–2624.
21. PERCIVAL, S.S., S.T. TALCOTT, S.T. CHIN, A.C. MALLAK, A. LOUNDS-SINGLETON, J. PETTIT-MOORE, Neoplastic transformation of balb/3t3 cells and cell cycle of hl-60 cells are inhibited by mango (*Mangifera indica l.*) juice and mango juice extracts, *J. Nutr.*, 2006, **136**, 1300–1304.
22. REN, W., Z.QIAO, H. WANG, L. ZHU, L. ZHANG, Flavonoids: promising anticancer agents, *Medicinal Research Reviews*, 2003, **23,** 519–534.
23. SHEFER, G., T.A. PARTRIDGE, L. HESLOP, J.G. GROSS, U. ORON, O. HALEVY, Low energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells, *J. Cell Sci*., 2002, **115,** 1461–1469.
24. TUNER, J., L. HODE, *Laser Therapy*, Prima Books, Grängesberg, 2002.
25. VIJAYABABU, M.R., A. ARUNKUMAR, P. KANAGARAJ, J. ARUNAKARAN, Effects of quercetin on insulin-like growth factors (IGFs) and their binding protein-3 (IGFBP-3) secretion and induction of apoptosis in human prostate cancer cells, *J. Carcin.*, 2006, **5,** 10–18.
26. VO-DINH, T., ed., *Biomedical Photonics Handbook*, CRC Press, Boca Raton, 2003.
27. WENZEL, U., A. HERZOG, S. KUNTZ, H. DANIEL, Protein expression profiling identifies molecular targets of quercetin as a major dietary flavonoid in human colon cancer cells, *Proteomics*, 2004, **4,** 2160–2174.
28. WHEELAND, R.G., History of lasers in dermatology, *Clin. Dermatol*., 1995, **13**, 3–10.
29. WILLIAMS, R.J., J.P. SPENCER, C. RICE-EVANS, Flavonoids: antioxidants or signalling molecules?, *Free Radic Biol Med.*, 2004, **36,** 838–849.
30. WONG-RILEY, M.T.T. *et al.*, Photobiomodulatiom directly benefits primary neurons functionally inactivated by toxins, *J. Biol. Chem.*, 2005, **280**, 4761–4771.
31. YANG, C.S., J.M. LANDAU, M.T. HUANG, H.L. NEWMARK, Inhibition of carcinogenesis by dietary polyphenolic compounds, *Annu. Rev. Nutr*., 2001, **21,** 381–406.
32. YANG, J.H., T.C. HSIA, H.M. KUO, P.D. LEE CHAO, C.C. CHOU, Y.H. WEI, J.G. CHUNG, Inhibition of lung cancer cell growth by quercetin glucuronides via G2/M arrest and induction of apoptosis, *Drug Metabolism and Disposition*, 2005, **90**, 9556.
33. YUAN, ZHI-PING *et al*., Liposomal quercetin efficiently suppresses growth of solid tumors in murine models, *Clinical Cancer Research*, 2006, **12**, 3193–3199.