CELLULAR STRESS AND DNA DAMAGE INDUCED BY EXCITATION VISIBLE LIGHT IN CONFOCAL MICROSCOPY

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BACKGROUND. When visible light of the intensity used in standard confocal fluorescence imaging is focused inside the cell nucleus, various proteins responsible for repair of several types of DNA lesions, including single and double strand DNA breaks, are recruited to this region of chromatin. This recruitment is interpreted as evidence of the existence of a DNA damage which must have been inflicted by exposure to the laser beam [1]. Induction of such damage is surprising in view of the fact that DNA shows only negligible absorption of visible light.

AIM. In order to provide more information regarding the unexpected induction of DNA damage by visible light in a confocal microscope, we studied various signs of cellular stress and damage following exposure to the light emitted by lasers typically used in confocal microscopes.

METHODS. We assessed the adverse effects induced by light in nonstained, nontransfected and transfected cells in G1, S or G2 phase of the cell cycle, by detecting accumulation of XRCC1 [2] and 53BP1 DNA repair proteins, and monitoring cell plasma membrane blebbing and integrity, cell cycle delay and cell death.

RESULTS. Doses of blue light below 35 μJ brought about accumulation of DNA repair factors, but no detectable signs of cellular stress, however the dose of 7 mJ induced membrane blebbing. The doses of 7-19 mJ induced cell cycle delay or arrest of cells in G1 or S phase. Doses above 140 mJ induced cell death within 1 to 4 hours after exposure.

CONCLUSION. Even relatively low doses of visible light, that are often used in standard confocal imaging (and are significantly lower than the doses used in FRAP experiments, STED super-resolution microscopy or manipulating cell components by laser tweezers) induce various signs of cellular stress, and cause DNA and membrane damage and cell death. The fact that DNA damage is readily inflicted by laser beams used for excitation of fluorescent probes and tags should be taken into account when interpreting observations made by confocal microscopy techniques.

REFERENCES

[1] KJ. Solarczyk, M. Zarębski, JW. Dobrucki, „Inducing local DNA damage by visible light to study chromatin repair”, *DNA Repair (Amst)*, **11(12)**, 996-1002 (2012).
[2] KJ. Solarczyk, M. Kordon, K. Berniak, JW. Dobrucki, „Two stages of XRCC1 recruitment and two classes of XRCC1 foci formed in response to low level DNA damage induced by visible light, or stress triggered by heat shock”, *DNA Repair (Amst)*, **37**, 12-21 (2016).