X-ray assisted photodynamic therapy for pancreatic cancer

M. Stepić[1,*], M. Živanović[2], M. D. Nešić[1], J. Žakula[3], I. A. Popović[1], V. Ž. Ralić[1], L. Korićanac[3], P. Lobachevsky[4], A. N. Bugay[4], O. O. Vinogradova[4], V. S. Vinogradova[4] and M. Ž. Petković[1]

[1]*COHERENCE Centre, Department of Atomic Physics, VINČA Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, 11000 Belgrade, Serbia*
[2]*Department of Radiation and Environment Protection, VINČA Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, 11000 Belgrade, Serbia*
[3]*Department of Molecular Biology and Endocrinology, VINČA Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, 11000 Belgrade, Serbia*
[4]*Laboratory of Radiation Biology, Joint Institute of Nuclear Research, 141980 Dubna, Moscow Region, Russia*

***e-mail: mstepic@vin.bg.ac.rs**

GOAL

Pancreatic cancer is among the most deadly forms of deep-seated cancer globally, with one of the lowest survival rates. In this work, we experimentally examined the viability of MIA PaCa-2 pancreatic cancer cells as a function of the concentration of the photosensitive molecule protoporphyrin IX (pPIX) alone or doped with a rare-earth element, Gd (pPIX Gd). Also, the *in-vitro* viability of both untreated and treated MIA PaCa-2 cells under irradiation with X-rays of different doses is explored.

INTRODUCTION

Conventional treatments for cancer, such as chemotherapy, radiotherapy, and surgical resection, have shown limited healing efficacy and may cause severe side effects, poor targeting, and drug resilience for monotherapies, which strongly restrict their clinical application. Thus, various combinatorial strategies, such as photodynamic therapy (PDT), are investigated in detail to combat cancer. PDT [1] is a controllable, non-invasive, and non-cumulative two-step treatment that combines a nontoxic and biocompatible photosensitiser (PS), oxygen, and light to annihilate tumour cells and tissues. Upon light activation, the PS generates reactive oxygen species (ROS), which selectively destroy the targeted malignant tissue.

The deep-seated tumours, such as those in the lungs, pancreas, ovaries, colorectum, and kidneys, are challenging to treat with PDT because visible or near-infrared light has a tissue penetration depth of less than 3 cm, thus limiting PDT treatment to superficial tissues. This obstacle may be eliminated by X-PDT, a PDT based on X-ray irradiation, which penetrates deeper than visible or infrared light [2]. X-PDT utilises a scintillator (e.g., lanthanides) to convert external X-ray photons into visible light photons, which in turn activate the PS to trigger PDT-mediated processes within the tumour tissue.

CYTOTOXICITY

MIA PaCa-2 cells from American Type Culture Collection (ATCC, Manassas, VA, USA) are seeded in flat-bottomed 96-well microtiter plates and incubated overnight in 90 µl Dulbecco's Modified Eagle's Medium (DMEM) (37 °C, 5% CO_2). Different amounts of prodrug were dissolved in DMSO and additionally diluted in PBS. 10 µl of such solution was poured gently in seeded wells filled with DMEM. Cytotoxicity of the pPIX and pPIX Gd was measured by Sulforhodamine B (SRB) assay 48 h after treating the cells with different concentrations of prodrugs.

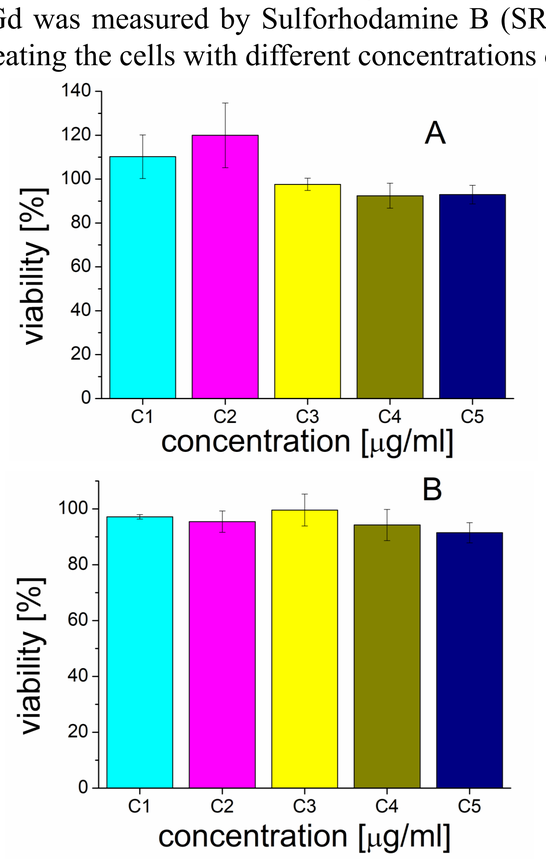

Figure 1. Viability of MIAPaCa-2 cells treated with pPIX (A) and pPIX Gd (B). Concentrations are C1=50 µg/ml, C2=25 µg/ml, C3=12.5 µg/ml, C4=6.25 µg/ml and C5=3.125 µg/ml, respectively.

X-RAY IRRADIATION

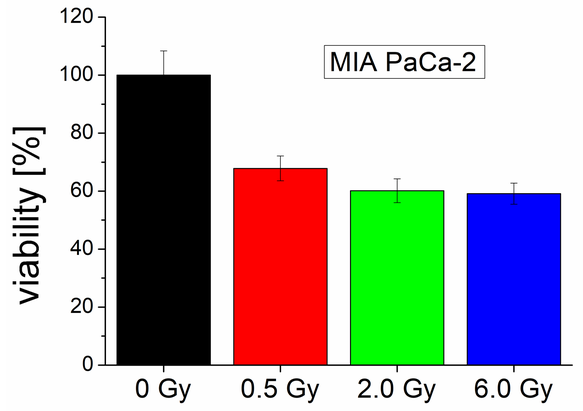

Figure 2. Effects of X-ray irradiation on MIAPaCa-2 cells.

96-well microtiter plates seeded with MIA PaCa-2 cells were irradiated in dark room by different doses of X-ray from a constant potential X-ray generator (Hopewell Designs X80-225 kV, Alpharetta, Georgia, USA).

MIA PaCa-2 cell viability was experimentally determined to be in the range 60-68%, using the SRB assay. The absorbance was measured in quadruplets for each dose at 550 nm with a reference wavelength of 690 nm in a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, PerkinElmer, Turku, Finland).

COMBINED TREATMENT (X-PDT)

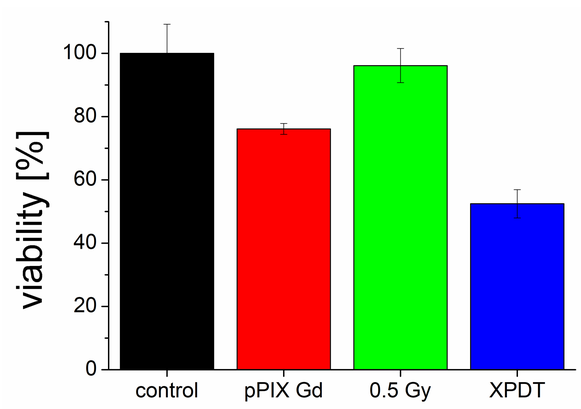

Figure 3. Viability of MIAPaCa-2 cells: control (black), treatment (red), X-ray irradiation (green), and combined treatment and irradiation (blue).

Firstly, for this experiment, we have used concentration of pPIX Gd which resulted in rather low cytotoxicity of MiaPaCa-2 cells 48 hours after the treatment (the mortality rate ≈ 26%).

The irradiation condition (a single dose of 0.5 Gy per well) is chosen as the one with the weakest influence on untreated pancreatic cancer cells (the mortality rate ≈ 11%).

Finally, we irradiated MIA PaCa-2 cells previously treated with pPIX Gd and observe a notable synergistic effect, resulting in total cell viability reduced to ≈ 51%.

MORPHOLOGICAL CHANGES IN CANCER CELLS

Monitoring the morphological changes in irradiated cancer cells or cancer cells treated with cytotoxic drugs is often used to elucidate their individual or mutual influence on tumour. Control MIA PaCa-2 cells (Fig. 4A) display normal morphology, exhibiting both typical round and spindle shapes, with loosely associated clusters present. Irradiated cells (0.5 and 6 Gy) retain their characteristic shape but are less dense than the control cells (Figs. 4B, C). The decrease in density is more pronounced when 6 Gy is applied. Cells treated with pPIX or a combination of pPIX and gadolinium show a shape and density similar to that of control cells (Figs. 4 D, E). The pPIX particles are clearly visible in the images.

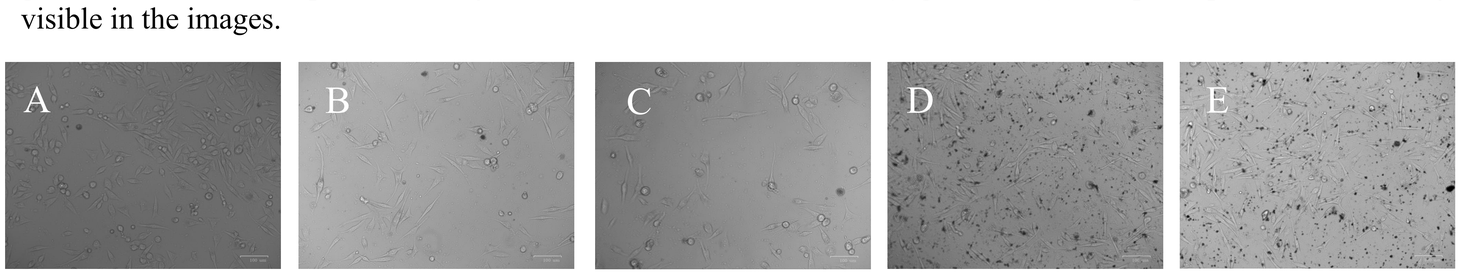

Figure 4. Morphological changes in MIAPaCa-2 cells: untreated control (A), after 0.5 Gy irradiation (B), after 6.0 Gy irradiation (C), after treatment with pPIX (D) and after treatment with pPIX Gd (E).

CONCLUSION

Seminal experimental results suggest that pancreatic MIA PaCa-2 cells may be quite efficiently killed by the joint action of confined, low-level X-ray radiation and the photosensitive molecule protoporphyrin IX doped with Gd. Further optimization of used X-ray doses and prodrug concentration is needed.

LITERATURE

[1] M. Matijević et al., Photochem. Photobio. Sci. 2036, 1087 (2021).

[2] O. Sahin et al., Sci. Adv.11, eadr 4008 (2025).

ACKNOWLEDGEMENTS

The Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract No. 451-03-136/2025-03/ 200017) and bilateral JINR-Serbia grant P_210.