Advances on photodynamic therapy through new pyridine-fused diphenylchlorins as photosensitizers for melanoma treatment

J. Dias-Ferreira,1,2,* Nelson A.M. Pereira,3 Mafalda Laranjo,1,4,5 Marta Pineiro,3 João Casal–Lopes,1,4,5 Ana Margarida Abrantes,1,4,5 Teresa M.V.D. Pinho e Melo,3 Maria Filomena Botelho1,4,5

1 Biophysics Unit, Faculty of Medicine of University of Coimbra, Azinhaga de Santa Comba, Celas 3004-548, Coimbra, Portugal
2 Faculty of Pharmacy of University of Coimbra, Azinhaga de Santa Comba, Celas 3004-548, Coimbra, Portugal
3 CQC and Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal
4 CIMAGO –Center of Investigation in Environment, Genetics and Oncobiology, Faculty of Medicine of University of Coimbra, Azinhaga de Santa Comba, Celas, 3004-548 Coimbra, Portugal
5 CNC.IBILL, University of Coimbra, 3004-535 Coimbra, Portugal
6 Servicio de Radioterapia, Centro Hospitalar e Universitário de Coimbra, Praceta Mota Pinto, 3000-075 Coimbra, Portugal

E-mail address: j.dias.ferreira@outlook.pt (J. Dias-Ferreira).

Aim: Assessment of cytotoxicity of four new photosensitizers intended for photodynamic therapy (PDT) against melanoma cells (A375 cells).

Introduction: Melanoma is the rarest form of skin cancer. PDT combines a photosensitizer with light culminating in the production of reactive oxygen species leading to cellular death. A new type of stable 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused tetraphenylchlorins1 proved to be very active as photodynamic agents. Thus, looking for a new generation photosensitizers with optimized properties for PDT we synthesized new diphenylchlorins.

Methods: The human melanoma cell line A375 was seeded in 48 well plates. The photosensitizers NAMP103A, NAMP103B (the tetraphenylchlorins monoester), NAMP263A and NAMP263B (the tetraphenylchlorins alcohol) were administered ranging 5 nM to 10 μM. Irradiation was performed after 24 h (λ < 560 nm). MTT and SRB assays as well as flow-cytometry were performed 24 h after the PDT.

Results: MTT assay results allowed to obtain dose–response curves and to calculate the concentration that inhibits the cultures by 50% (IC50). Phototoxicity (10 J) was dependent on the chlorines concentration. Moreover, NAMP263B was significantly more cytotoxic than NAMP103A (p = 0.037) and NAMP103B (p = 0.042). From SRB assay we verified that with a 125 nM concentration the NAMP103A, NAMP103B, NAMP263A and NAMP263B produce a cellular viability of 36.9%; 33.2%; 18.3% and 18.8%, respectively. Flow cytometry studies confirmed the decrease of viability associated with cell death by apoptosis and necrosis. Loss of mitochondrial membrane potential, apoptosis hallmark, was also observed. An imbalance of ROS, namely superoxide anion and peroxides, was observed for all photosensitizers studied with an exhaustion of antioxidant intracellular defenses (GSH). At low PS concentrations (5 nM), metabolic activity was variable with light energy (5 J, 10 J and 20 J) with lower values for higher fluorescence. Dark toxicity studies revealed photosensitizer dependence of irradiation.

Conclusion: We hereby conclude that the photosensitizers are indeed very promising, which rouses plans for following proceedings to verify in vivo outcome.

Acknowledgements: FCT Portugal: Project PTDC/QEQ-MED/0262/2014, Strategic Project Pest CNC.IBILL UID/NEU/04539/2013 and Strategic Project Coimbra Chemistry Centre UID/QUI/00313/2013, COMPETE-FEDER.1,2

References

http://dx.doi.org/10.1016/j.pbj.2017.07.119

Angiogenesis and inflammation at the crossroads between diabetes and cancer

R. Rocha,1,2,* J. Rodrigues,1 I. Gullo3,4,5,6 G. Gonçalves3,4, J. Pedro1, D. Carvalho4,7 F. Carneiro3,4,5,6, F. Soares1,4, S. Andrade1,4,5

1 Unit of Biochemistry, Department of Biomedicine, Faculty of Medicine, University of Porto, Portugal
2 Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal
3 Institute of Molecular Pathology and Immunology at the University of Porto (IPATIMUP), Porto, Portugal
4 Instituto de Investigação e Inovação em Saúde (I3S), University of Porto, Porto, Portugal
5 Department of Pathology, Centro Hospitalar de São João, Porto, Portugal
6 Department of Pathology, Faculty of Medicine of the University of Porto (FMUP), Porto, Portugal
7 Department of Endocrinology, Centro Hospitalar de São João, Porto, Portugal

E-mail address: anaaritarocha@ua.pt (R. Rocha).

Aim: To study fibrosis, angiogenesis, oxidative stress and inflammation markers in diabetic and non-diabetic patients with gastric cancer (GC).

Introduction: Type 2 Diabetes mellitus (DM2) is a major health problem, with 415 million people diagnosed worldwide.1 Evidence regarding its association with various types of cancer has been reported, including GC.2 Some hypotheses have been suggested to explain how DM2 could enhance the risk of cancer development, such as hyperglycemia, hyperinsulinemia, oxidative stress, vascular disturbances and a chronic low inflammation state.3–5

Gastric cancer (GC) is the fifth most common cancer worldwide and ranks as the third leading cause of cancer-related death.6 GC is frequently associated with infection by Helicobacter pylori and inflammation plays a central role in the carcinogenic process. Such chronic inflammatory state, linked with angiogenesis imbalance, oxidative stress and metabolic signaling, suggests that also DM2 might be a major risk factor in initiation and progression of GC, demanding further investigation.

Methods: A series of GC from DM2 (n = 22) and nonDM2 (n = 21) patients were studied. Immunohistochemistry (IHC) using antibodies against CD31 and 3-Nitrotyrosine was performed, to assess density of vessels and oxidative stress status. Histochemical staining with Sirius red was performed to determine the percentage of fibrosis in the tumor and non-neoplastic adjacent mucosa. Based on assessment of tumor inflammatory cell infiltrate and tumor stroma...
Results: Diabetic patients presented a significant higher glycaemia than the control patients (190.1±13.6 mg/dL vs 98.2±13.6 mg/dL, p < 0.001, respectively). Decreased survival rates were observed in diabetic patients (611.5 vs 916.0, p = ns). Tumours exhibited increased fibrosis relatively to the adjacent mucosa in both groups and diabetic patients (N: 9.362±1.337; T: 12.29±1.407) presented higher fibrosis levels than the non-diabetic patients (N: 7.165±1.017; T: 10.97±1.076).

Conclusion: Expected results: Identifying the distinct features that characterize GC of DM2 patients compared to non-diabetic patients (namely fibrosis, angiogenesis, inflammation, and oxidative stress biomarkers) will enable to study this subset of GC patients and unravel key mechanisms behind the relationship between DM2 and GC.

Acknowledgements: Funding: This work was supported by the project Diabetes & obesity at the crossroads between Oncological and Cardiovascular diseases – a system analysis NETwork towards precision medicine (DOCnet) – A multi-omics approach to decipher diabetes-related molecular targets in cancer: a step towards precision medicine. NORTE2020 – “Programa Operacional Regional do Norte” (NORTE-01-0145-FEDER-000003) (Jan 2016-Dec2018).

References


http://dx.doi.org/10.1016/j.pbj.2017.07.120