Development and validation of an *in vitro* fluorimetric quantification method of ROS generation using the DCFH-DA probe to detect quickly the presence of a drug-induced oxidative stress

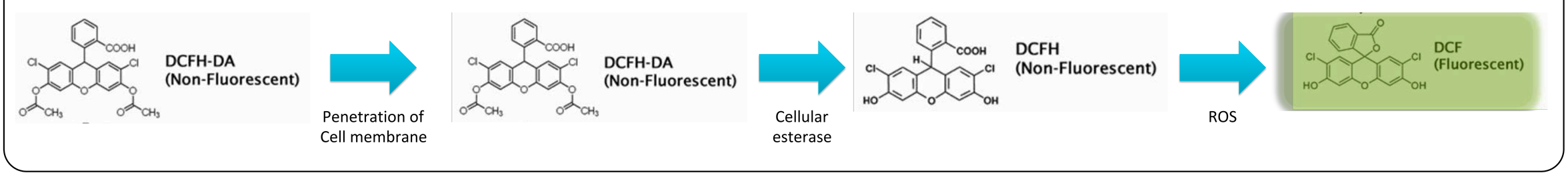
Matthieu Dallons, Vanessa Tagliatti
Department of Human Biology & Toxicology (Prof. J.M. Colet) - UMONS

Introduction

Oxidative stress is an important cellular mechanism implicated in many drug adverse effects. Oxidative stress occurs when there is an imbalance between the generation and the degradation of radial species in an organism. The majority of these radicals involve the oxygen atom and are named as reactive oxygen species (ROS). ROS are able to damage biological tissues and cellular components by oxidation of lipids, proteins and nucleic acids *(1)*. Oxdative stress is a key toxicological mechanism implicated in doxorubicin cardiotoxicity. It is known that this anticancer drug induces an oxidative stress in cardiomyocytes by the generation of ROS through a redox cycle and the formation of a doxorubicin-iron complex *(2,3)*. Therefore, the objective of this work was the optimization and the validation of a quickly *in vitro* fluorimetric quantification method of ROS using the dichloro-dihydro-fluorescein diacetate (DCFH-DA) probe in H9C2 cells for doxorubicin-induced oxidative stress assessment.

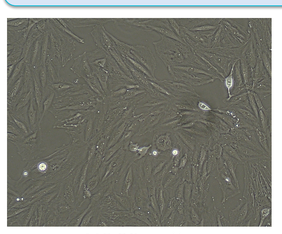
Principle of the DCFH-DA assay

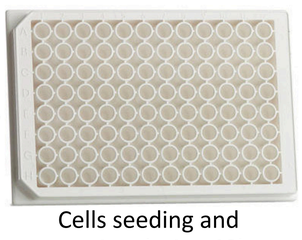
DCFH-DA is the most widely used probe for detecting intracellular H_2O_2 and oxidative stress. This probe is cell-permeable and is hydrolyzed intracellularly to the DCFH carboxylate anion, which is retained in the cell. Two-electron oxidation of DCFH results in the formation of a fluorescent product, dichlorofluorescein (DCF), which can be monitored by several fluorescence-based techniques (e.g., confocal microscopy, flow cytometry) *(4)*.



Development and validation of the method

Protocole


H9C2 cells

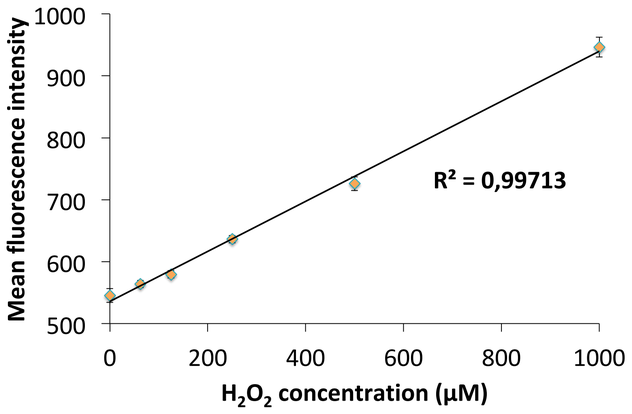

Cells seeding and
growing during 48h

Exposure to the drug and incubation

Addition of DCFH-DA probe and incubation


Fluorescence reading
at 490 nm (EX) / 510-570 nm (EM)

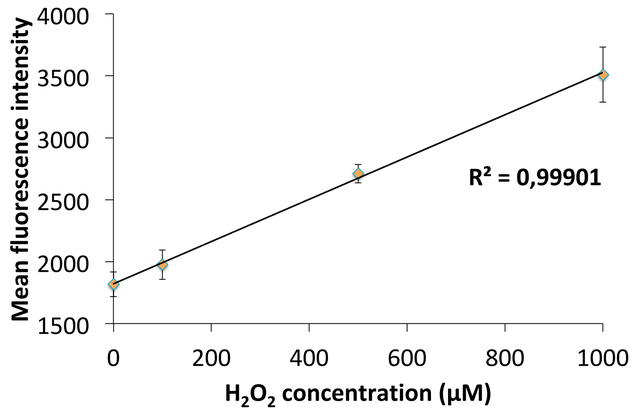
Linearity and variability of the method



Mean fluorescence intensity (+/-SEM) for different H_2O_2 concentrations without H9C2 cells, during 2 hours.

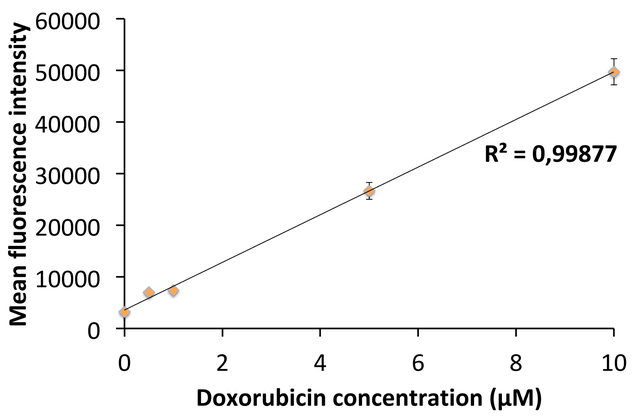
H_2O_2 concentration (µM)	Coefficient of variation (CV)
0	2,65 %
62,5	2,61 %
125	2,51 %
250	4,23 %
500	5,36 %
1000	6,03 %

Coefficient of variation of the fluorescence intensity measured for different H_2O_2 concentrations without any H9C2 cells.



Mean fluorescence intensity (+/-SEM) of H9C2 cells exposed to different H_2O_2 concentrations during 2 hours.

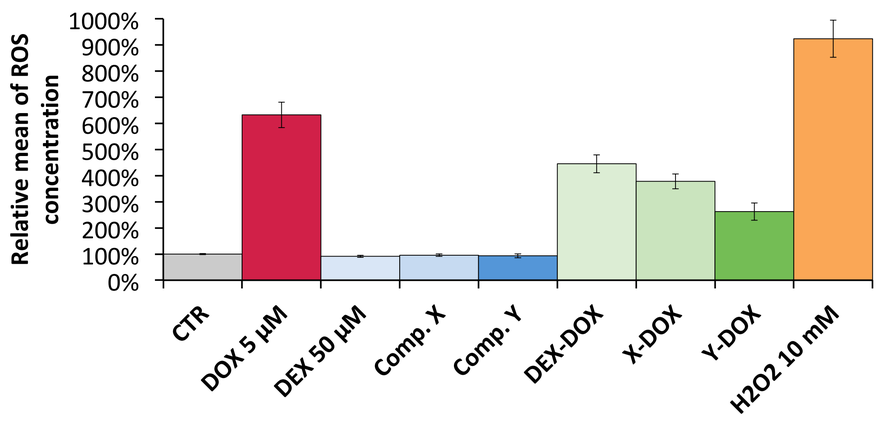
Application for the assessment of doxorubicin-induced oxidative stress in H9C2 cells



Mean fluorescence intensity (+/-SEM) of H9C2 cells exposed to different doxorubicine concentrations during 2 hours.

Doxorubicin concentration (µM)	Coefficient of variation (CV)
0	6,49 %
0,5	17,67 %
1	15,20 %
5	15,03 %
10	12,49 %

Coefficient of variation of the fluorescence intensity measured after H9C2 exposure to different doxorubicin concentrations during 2 hours.



Rleative mean of ROS concentration (+/-SEM) of H9C2 cells exposed to doxorubicine and different protective compounds during 2 hours.

References

1. Kelly KA, Havrilla CM, Brady TC, Abramo KH, Levin ED. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environ Health Perspect. 1998 Jul;106(7):375–84.
2. Damiani RM, Moura DJ, Viau CM, Caceres RA, Henriques JAP, Saffi J. Pathways of cardiac toxicity: comparison between chemotherapeutic drugs doxorubicin and mitoxantrone. Archives of Toxicology. 2016 Jun 25.
3. Chung W-B, Youn H-J. Pathophysiology and preventive strategies of anthracycline-induced cardiotoxicity. The Korean Journal of Internal Medicine. 2016 Jul 1;31(4):625–33.
4. Kalyanaraman B, Darley-Usmar V, Davies KJA, Dennery PA, Forman HJ, Grisham MB, et al. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radic Biol Med. 2012 Jan 1;52(1):1–6.

Université de Mons
Contact : Matthieu.DALLONS@umons.ac.be