Thyrocytes are particularly well protected against oxidative stress induced by H_2O_2

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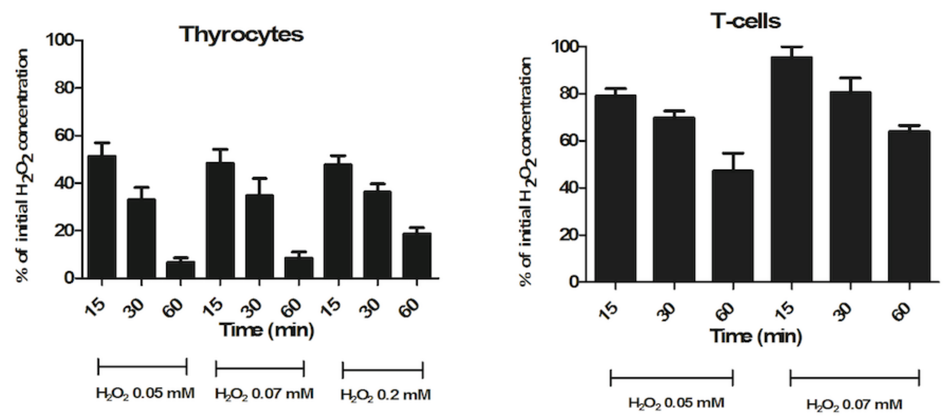
Background:
H_2O_2 produced in large quantities in the thyroid may play a role in the pathogenesis of thyroid nodules and cancer. *In vitro*, moderate amounts of H_2O_2 are able to cause similar DNA damage compared to irradiation and even to induce RET/PTC rearrangements.

Objective and hypotheses:
We compared the defence mechanisms against H_2O_2 and irradiation in human thyrocytes, T-cells and other cell types.

Method:
Human thyrocytes in primary culture were compared to other cell types: human T-cells in primary culture, a human thyroid epithelial cell line (Nthy-ori 3-1), non-transformed rat fibroblasts (F208) and a human myeloid cell line (PLB-XGCD) in terms of ability to degrade H_2O_2, glutathione peroxidase (GPx) activity, heme oxygenase-1 (*HMOX1*) expression, cell survival and capacity to repair DNA damage after H_2O_2 exposure or irradiation (Cs^{137} source).
H_2O_2 was measured in the medium by a sensitive fluorimetric assay. Cells were incubated overnight with BSO (Buthionine-sulfoximine, an indirect inhibitor of Glutathione peroxidase) before H_2O_2 or irradiation treatment. GSH peroxidase activity was measured for each cell type. qPCR were performed on cells after different treatments (H_2O_2, irradiation) to study regulation of the Heme oxygenase 1 (HMOX1) transcription. Alkaline COMET assay was used to measure total DNA damage after treatment with radiation or H_2O_2. Survival test were evaluated by MTS/PMS test and by FACS analysis

Results

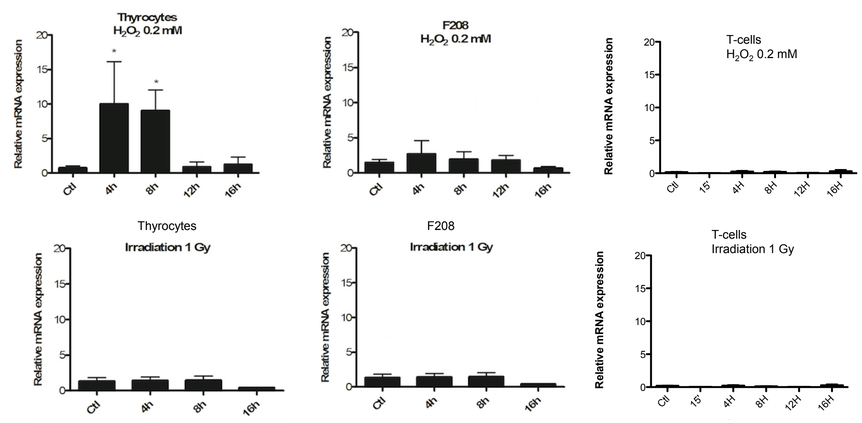
1) Thyrocytes are particularly efficient to degrade H_2O_2: Clearance of extracellular H_2O_2 added to the medium of thyrocytes and T-cells: 300.000 cells



100%: H_2O_2 measured in the same condition without cells

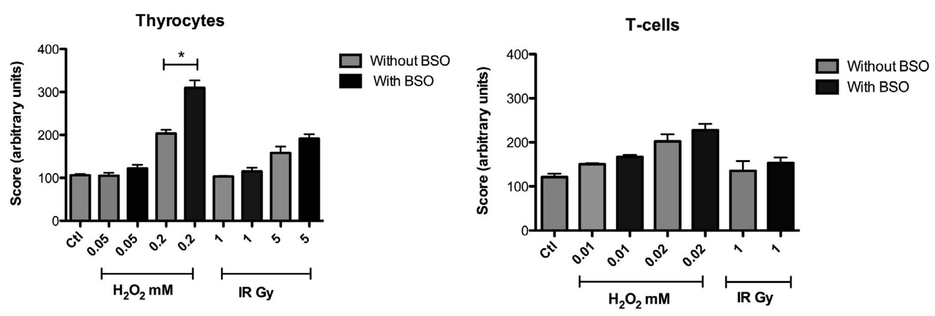
→ Thyrocytes degrades extracellular H_2O_2 more efficiently than other cell types

4) H_2O_2 induces the expression of another antioxidant enzyme in the thyrocyte: the Heme oxygenase 1: RT-qPCR of HMOX1



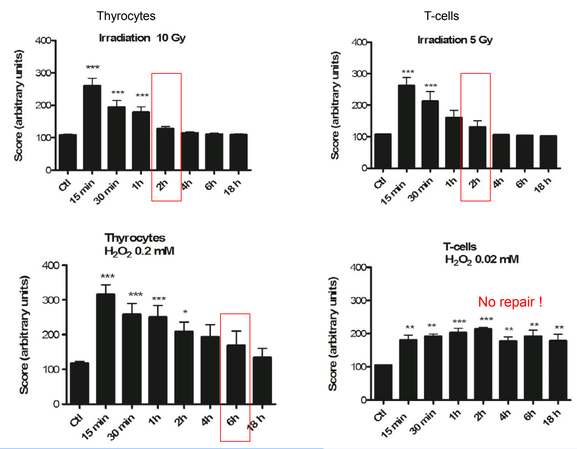
→ there is an upregulation of HMOX1 mRNA expression after 4 to 8 hours only in thyrocytes after H_2O_2 treatment. No effect of irradiation is observed

2)Gluthtione peroxidase protects thyrocytes against DNA damage induced by H_2O_2: Effect of BSO on global DNA damage



→ The detoxification by GPx is more efficient in thyrocyte than in T-cell

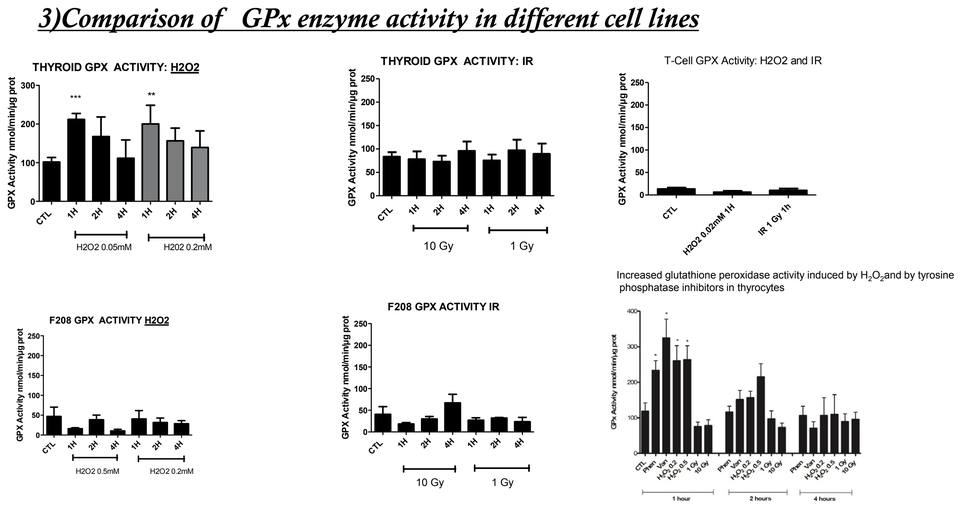
5) How fast is DNA damage repaired after H_2O_2 and irradition treatment ?



→ Damage caused by H_2O_2 is repaired much more slowly than those caused by irradiation

→ Unlike other cell types used in this work, T cells are unable to repair the damage caused by H_2O_2

3)Comparison of GPx enzyme activity in different cell lines



→ GPx activity is stimulated after a treatment with H_2O_2 after 1 hour and only in thyrocytes, no effect is observed after irradiation. Vanadate and bp V Phen mimic the effect of H_2O_2

Conclusion:

Thyrocytes rapidly degraded extracellular H_2O_2 and presented a low mortality rate after H_2O_2 exposure. Thyrocytes had the highest basal GPx activity which was stimulated by H_2O_2. This effect was mimicked by tyrosine phosphatase inhibitor treatment. Expression of HMOX1 mRNA was up-regulated by H_2O_2 in thyrocytes but not in the other cells. HMOX1 expression and GPx activity were unchanged after irradiation in all tested cell types. DNA damage caused by irradiation was more rapidly repaired than that caused by H_2O_2 in all investigated cells. T-cells did not repair DNA damage caused by H_2O_2

Thyrocyte have developed multiple mechanisms of protection against oxidative stress induced by H_2O_2. Our results suggest that deficiency of one of these mechanisms could promote the appearance of sporadic thyroid cancer. Due to their extreme sensitivity to H_2O_2, T-cells are probably not a good surrogate tissue to study individual susceptibility to H_2O_2.

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