USE OF A MODIFIED COMET ASSAY FOR GENOTOXICITY ASSESSMENT OF POLLUTANTS IN THREE FISH CELL LINES

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Introduction

Because of the increasing regulatory pressure regarding the assessment of environmental impact of chemicals, and in order to reduce whole organism testing, *in vitro* models are of growing interest. Fish cell lines constitute an alternative and easy to use vertebrate-based test system in aquatic ecotoxicology studies (Bols *et al.* 2005; Schirmer 2006). About one third of aquatic contaminants are suspected to interact with DNA structure and functions (Claxton *et al.*, 1998, Ohe *et al.*, 2004).
Primary DNA damage is frequently used to assess genotoxicity potential of pollutants *via* the Comet assay. In this work, we chose to use a modified version of the comet assay using a restriction enzyme, in order to increase its sensitivity, so that genotoxicity could be detected in environmentally relevant conditions. The **Fpg restriction enzyme** was used for its capacity to detect various lesions such as oxidation or alkylation, therefore broadening the type of DNA damage detected by the Comet assay.
The feasibility and the usefulness of such a modified Comet assay has been tested on three permanent fish cell lines, characterized by contrasted metabolic and growth capacities.

Materials and Methods

3 fish cell lines with contrasted metabolic capacity

RTG-W1 *(ATCC CRL-2523)*
Rainbow trout gill cells cultivated at 20° C in Leibovitz medium + 10% FBS

RTL-W1
Rainbow trout liver cells (Lee *et al.* 1993) cultivated at 20° C in Leibovitz medium + 10% FBS

PLHC-1 *(ATCC CRL-2406)*
Topminnow hepatocellular carcinoma cells cultivated at 30° C in Leibovitz medium + 10% FBS

Main issues

-Can the Fpg-modified Comet assay be carried out in those cell lines ?

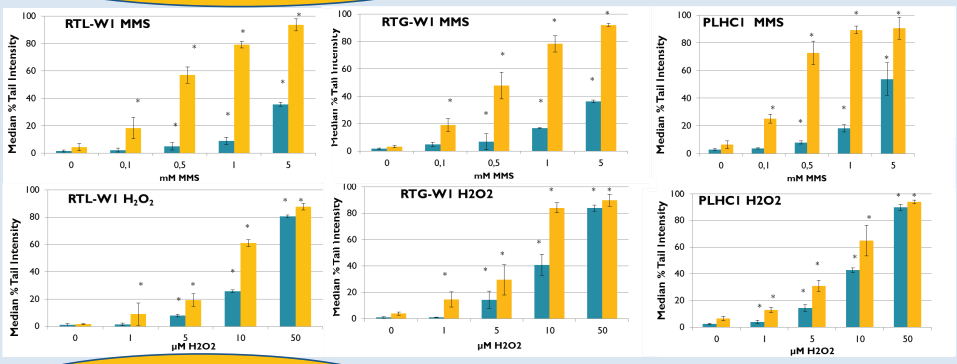
-What is the gain in sensitivity *vs* classic Comet assay?

-Does it allow a detection at environmentally relevant concentrations?

-What is the sensitivity of those models compared to whole organisms?

Results and Discussion

1-Testing the Fpg modified Comet assay protocol with model genotoxicants



*: statistically significant difference compared to control, Kruskall Wallis, p<0.05

LOW basal DNA damage for the three cell lines !

RTL-W1=RTG-W1<PLHC1

Median % Tail Intensity	No enzyme		With Fpg	
RTL-W1	1,22	0,65	3,33	2,03
RTG-W1	1,54	1,34	3,75	1,67
PLHC 1	3,28	0,95	7,67	2,59

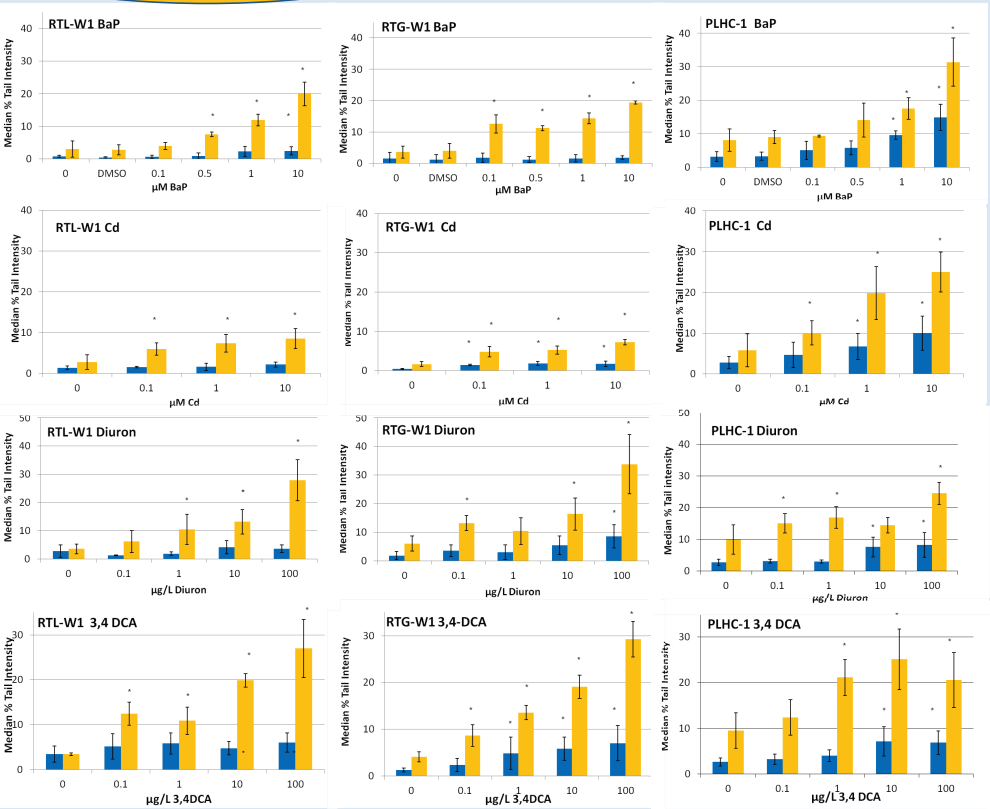
For the 3 cell lines:

-Similar dose-dependent response

-Statistically significant difference detected earlier with Fpg-modified Comet assay

-Modified Comet assay feasible but some restrictions when using PLHC1:
- *very sensitive to oxidative stress and culture conditions*
- *PLHC1 cells tend to aggregate*

2-Application to environmental genotoxicants



-BaP genotoxicity *detected with Fpg on the three cell lines*
-Cd, Diuron, 3,4-DCA (diuron metabolite) genotoxic response *increased with Fpg*

Concentration of the first significant genotoxic effect		RTG-W1	RTL-W1	PLHC-1	*Environmental concentrations*
Cd (µM)	Without enzyme	0.1	NS	1	
	Fpg	0.1	0.1	0.1	0,24-1,27 nM
	Sensitivity factor	1	+∞	10	
Diuron (µg/L)	Without enzyme	100	NS	10	
	Fpg	0.1	1	0.1	*0,2 to 5,4 µg/L*
	Sensitivity factor	1000	+∞	100	
3,4-DCA (µg/L)	Without enzyme	1	NS	10	
	Fpg	0.1	0.1	1	*0,3 to 8,9 µg/L*
	Sensitivity factor	10	+∞	10	
BaP (µM)	Without enzyme	NS	10	1	
	Fpg	0,1	0,5	1	3,96 to 39,6 pM
	Sensitivity factor	+∞	20	1	

Sensitivity factor: concentration leading to the first significant effect without enzyme/concentration leading to the first significant effect with Fpg . NS: no significant genotoxic effect; +∞: sensitivity factor when genotoxicity is detected with the Fpg assay only

	In vitro threshold standard assay	*modified assay*	*In vivo thres.*	*Test, species*	*References*
Diuron (µg/L)	*10 to 100*	*1*	*1-2*	*micronucleus test, zebrafish*	*Bony et al., 2008, 2010*
Cd (µM)	*1 to 10*	*0,1*	*0,89-8,89*	*micronucleus test, crussian carp*	*Arkhipchuk and Garanko, 2005*
BaP (µM)	*1 to 10*	*0,1 to 1*	*0,1*	*juvenile sea bass*	*Gravato and Santos, 2003*

CONCLUSION: *Modified Comet assay is achievable with those three cell lines. Increase in sensitivity ranges from 1 to 1000 depending on the cell line and the genotoxicant. Thus, cell lines are valuable models for genotoxicity testing via the Fpg-modified Comet assay, which allows to detect a larger array of lesions with an increased sensitivity, close to whole organism sensitivity, and at environnmentaly relevant concentrations.*

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