J. Chim. Phys. (1999) **96**, 143-146

Use of the comet assay to measure DNA damage in cells exposed to photosensitizers and gamma radiation

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RÉSUMÉ

Nous avons utilisé la méthode des comètes associée à des ADN-glycosylases, pour estimer les dommages de l'ADN dans des cellules après l'exposition à un rayonnement gamma ou après photosensibilisation par le bleu de méthylène ou l'acridine orange. Une calibration de la méthode des comètes a permis de mesurer le niveau basal et les taux de formation de 8-oxodGuo ainsi que le nombre de cassures de brins et de sites alcali labiles.

Mots-clé : irradiation γ, dommages de l'ADN, photosensibilisation, méthode des comètes

ABSTRACT

We used the comet assay associated with DNA-glycosylases to estimate DNA damage in cells exposed to gamma irradiation or photosensitized either with methylene blue or orange acridine. A calibration performed using γ irradiation allowed the measurement of the steady-state level and the yield of 8-oxodGuo as well as strand breaks and alkali-labile sites.

Key words : γ irradiation, DNA damages, photosensitization, comet assay

INTRODUCTION

We used the modified comet assay to detect DNA damage in cells exposed to either photosensitizers or gamma radiation. This method, as initially described [1], allows the detection of strand breaks (SSB +DSB) and alkali-labile sites (ALS). When associated with DNA repair glycosylases such as the *E. coli* formamidopyrimidine-DNA

glycosylase (Fpg) and the *E. coli* endonuclease III (Endo III) which recognize modified purines and pyrimidines, respectively, the method allows the detection of base damage [2]. It was possible using a calibration of the modified comet assay to measure very low levels of DNA damage such as 8-oxo-7,8-dihydroguanine (8-oxoGua). Indeed, up to now, the artefactual oxidation generated during the DNA extraction and subsequent work-up required for the usual techniques (HPLC-EC, GC-MS) made measurement of such low amounts of base damage not possible [3].

MATERIALS AND METHODS

THP-1 monocytes were exposed to γ–rays as already reported elsewhere [4]. For photosensitization reactions, 0.02 OD of methylene blue (MB) or orange acridine (OA) were added to the cell suspension. Visible light was generated by a 500 Watt halogen lamp. The irradiation times ranged from 0 to 2 min and from 0 to 20 min for the comet and HPLC-EC assays, respectively. The distance between the lamp and the sample was 25 cm. Control cell suspensions that contained MB or OA were kept in the dark. The protocols of the alkaline single-cell gel electrophoresis and of the DNA extraction have already been described in [4].

RESULTS AND DISCUSSION

The results obtained on cells either exposed to gamma irradiation or photosensitized with orange acridine showed an increase in the tail moment of 7.85 a.u. (arbitrary units of tail moment)/Gy , and 28.8 a.u./min of irradiation, respectively. In contrast, when methylene blue was used, no increase of the tail moment was observed. The background level of SSB + DSB + ALS was estimated to be, with the three agents, between 16 and 20 a.u. (Figure 1a).

The comet assay was modified as described by Collins *et al.* [2]. In this respect, Fpg was used to convert 8-oxodGuo into additional strand breaks. It was thus observed an increase in the tail moment (2.85 a.u./Gy) of the cells exposed to γ radiation. For the

photosensitized cells, we induced a saturation phenomenon consisting of a constant value of the tail moment at the level of 70 a.u. irrespective of the duration of the irradiation. Moreover, this increase was observed, even for the controls. This is likely to be explained by the saturation of the enzymatic response suggesting a very high yield of Fpg sensitive sites. Indeed, the oxidizing promoting effect of MB and OA was confirmed by the measurement of significant amounts of 8-oxodGuo by HPLC-EC. Results are presented in (Figure 1 b).

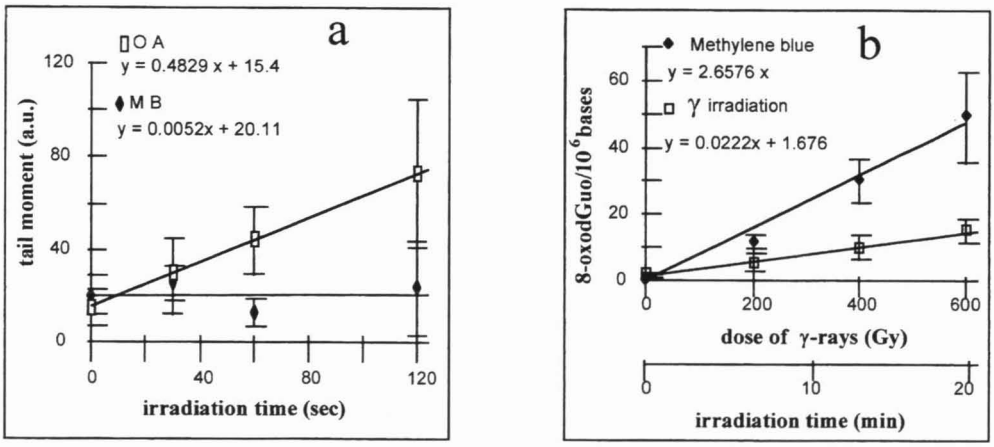

Figure 1: a) tail moment as a function of the dose in cells photosensitized with either methylene blue or orange acridine; b) measurement of 8-oxodGuo by HPLC-EC

To express a.u. of tail moment in terms of DNA lesions, attempts were made to calibrate the comet-Fpg assay by measuring 8-oxodGuo by HPLC-EC [4]. It was thus estimated that 1 8-oxodGuo/10^6 bases correspond to an increase of 127 a.u. of the tail moment as revealed by Fpg. It may be assumed that 8-oxodGuo represents, at least partly the residues sensitive to Fpg. This was used to compare DNA damage produced by either MB and OA in the presence of visible light or by gamma irradiation.

Table I: DNA lesions /10^6 bp in cells exposed to either γ radiation or to photosensitizers

	Fpg (-)	Fpg (+)	Fpg (-)	Fpg (+)	Fpg (-)	Fpg (+)
	methylene blue		orange acridine		γ irradiation	
Background level	0.15	0.60*	0.11	0.60*	0.26	0.18
Yield/Gy					0.12	0.044
Yield/min	0	0	0.45	0		

However with these preliminary results, we are not able to explain the high value of the tail moment observed in control cells treated with either MB or OA. The saturation response observed with these two photosensitizers suggest that: 1) The modified comet assay is a very sensitive method (for the same range of irradiation time, no increase of 8-oxodGuo is detected by HPLC-EC) 2) Ionizing radiation produces very low quantities of 8-oxodGuo, if compared with photosensitizers.

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