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Quantification and repair of psoralen-induced interstrand crosslinks in human cells

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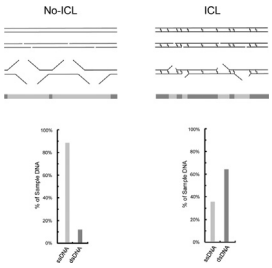
GRAPHICAL ABSTRACT



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ABSTRACT

Bi-functional alkylating agents that cause crosslinks are commonly used in chemotherapy. However, there is no conclusive knowledge for human cells regarding the number of induced interstrand crosslinks (ICLs) and the unhooking rate when the lesion is removed from one of the DNA strand. Using a newly developed method, we quantified the number of induced ICLs for the five furocoumarins; psoralen, 5-methoxypsoralen, 8-methoxypsoralen, tri-methoxypsoralen and angelicin. In quantitative terms, the results were in agreement with the values found by others. In kinetic studies using mammalian cells, we found that half of the psoralen-induced ICLs were unhooked within 2.5 h. The rate in normal human diploid fibroblasts was found to be 20,000 ICLs/h/cell. In comparison to survival, 2500 ICLs per cell led to 50% toxicity, indicating that the unhooking of the ICLs is not the crucial step for ICL tolerance. Surprisingly, only 3500 ICLs per cell corresponded to a significant delay in the replication fork elongation. The results indicate involvements of additional pathway(s) for the delay since the effect on replication elongation could be monitored when only 10% of the replication forks encounter an ICL.

Introduction

DNA crosslinking agents are commonly used in chemotherapy and are naturally present in fruits and vegetables. However, knowledge regarding the biological effects and repair of lesions caused by cross-linking agents in human cells remains unclear. DNA crosslinking agents are commonly used in cancer chemotherapy and in the treatment of psoriasis. In addition to the production of excess monoadducts, DNA crosslinking agents can also generate intra- and interstrand crosslinks (ICLs) in DNA due to their dual reactivity. ICLs pose a serious threat to the cells because they prevent replication and transcription. Because a template is not available on the complementary strand due to crosslinking, the repair of these lesions becomes complex. Although ICLs are considered to be the most hazardous type of lesions to the cell, the understanding of how ICLs are induced and repaired in mammalian cells is limited. Several models for ICL repair have been proposed, and the majority of these models suggest the involvement of nucleotide excision repair (NER), homologous recombination (HR) and/or trans-lesion synthesis (TLS) and Fanconi anemia (for recent reviews, see (Crossan and Patel, 2012; Cybulski and Howlett, 2011; Deans and West, 2011; Ho and Scharer, 2010; Muniandy et al.,

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2010; Sengerova et al., 2011; Wood, 2010). It remains unclear whether all proteins in NER repair are involved in ICL repair or only individual NER repair proteins have dual functions and operate separately from the rest of the NER machinery. The first unhooking event when the lesion is removed from one of the DNA strands has been proposed to include a role for XPF-ERCC1, possibly in coordination with other proteins (e.g., MUS81-EME1, FAN1 and/or SLX1-SLX4) (Andersen et al., 2009; Fekairi et al., 2009; Munoz et al., 2009; Svendsen et al., 2009).

Furocoumarins are a family of planar tricyclic compounds that are naturally occurring in edible plants, and psoralen is the most intensively studied member of this family. Psoralen can be absorbed by cells and has both toxic and mutagenic properties (Cimino et al., 1985; Ostertag et al., 2002). To become DNA reactive, psoralen must first undergo photo activation by UVA light (315–400 nm), after which it reacts with the 5,6-double-bonds of thymine to form covalent bonds (Serrano-Perez et al., 2008; Smith et al., 2004; Spielmann et al., 1995). The initial activation enables the molecule to react with one of the two sides of the DNA and can lead to the formation of a monoadduct. Following monoadduct formation a second photon may be absorbed activating the other end of the molecule to react with a base on the opposite strand, thereby forming an ICL (Serrano-Perez et al., 2008). The ability to form an ICL due to the second activation depends on which side of the molecule the first binding occurred (Serrano-Perez et al., 2008). Psoralen forms up to 40% ICLs among the total DNA adducts, therefore making it a valuable agent to study the biological effects from ICLs (Brendel and Ruhland, 1984). Another furocoumarin, angelicin, has an angular conformation and therefore can only form monoadducts (Kittler et al., 1980).

Previous studies on the damage response and repair of ICLs in mammalian cells have been mostly limited to relative studies, although investigations have been performed to estimate the number of ICLs in subcellular DNA induced by different furocoumarins (see Table 1). Thus, there is a need to develop an assay for quantifying the number of induced crosslinks and a fast and easy method to study the repair kinetics of ICLs in the intact genome of human cells.

Our assays for monitoring the induction and unhooking kinetics of ICLs in human cells partially met this objective. The method presented here is based on the technique of alkaline DNA unwinding (ADU) (Erixon and Ahnstrom, 1979), in which an alkaline solution will cause the DNA to start to unwind from introduced strand breaks. If the unwinding DNA encounters a DNA-DNA ICL, the unwinding will halt and be unable to proceed further, thereby protecting the DNA from further unwinding.

Using this ADU-based method that is capable of quantitatively analyzing psoralen-induced ICLs in mammalian cells, we observed that both the induction and unhooking of ICLs in two different human cell lines were similar. These cells were shown to have an unhooking capacity of approximately 20,000 ICLs per hour, whereas only 2500 ICLs per cell were required for lethality.

Table 1
A comparison of the estimated level ICL induction by various furocoumarins reported in published data.

Psoralen	5-Metoxy-psoralen	8-Metoxy-psoralen	TMP	Activation (UVA kJ/m^2)	Concentration (μM)	Method	Cell type	References
1.29 (R^2 = 0.99)	9.14 (R^2 = 0.94)	2.08 (R^2 = 0.96)	26.4 (R^2 = 0.99)	20–120 kJ	0–1	Alkaline DNA unwinding	Human fibroblasts	Vare et al. (2014)
N/A	3.63 (R^2 = 0.99)	2.67 (R^2 = 1)	19.4 (R^2 = 0.95)	1–2.4 kJ	1	Alkaline DNA unwinding	Chinese hamster cells	Dardalhon and Averbeck (1988)
N/A	N/A	3.12 (R^2 = 0.98)	N/A	5–50 kJ	2.7	LC–MS/MS	Human melanoma skin cancer cells	Cao et al. (2008)
N/A	N/A	3.83 (R^2 = 0.63)	N/A	5–100 kJ	2.7	LC–MS/MS	Human melanoma skin cancer cells	Lai et al. (2008)
N/A	N/A	0.63 (R^2 = 0.82)	N/A	19 kJ	75/150	Liquid scintilation	Leukocytes	Grass et al. (1998)

Data calculated to amount of ICLs per μM and kJ/m^2 of UVA activation.

Materials and methods

Cell lines

In this study, we used primary human fibroblast VH10 cells obtained from the foreskin of a 10-year old boy (Robichova et al., 2004) used in passages between 10 and 17. In all of the experiments VH10 cells were grown exponentially in F12 nutrient mixture (Ham) containing 9% FBS and 90 U/ml penicillin and streptomycin. During labeling, Dulbecco's modified Eagle's medium containing 1 g/l glucose (DMEM) was used instead of the F12 nutrient mixture. HAEB cells, derived from human embryonic lung tissue, were grown in DMEM. Psoralen, 5-methoxypsoralen, 8-methoxypsoralen, tri-methoxypsoralen, angelicin, cytosine arabinoside (AraC), and hydroxyurea (HU) were purchased from Sigma-Aldrich (Sweden). ^{3}H-TdR (37 MBq/ml) was purchased from Perkin Elmer, Stockholm, Sweden.