

DNA damage induced by hydrogen peroxide treatment at 4°C and 37°C in murine lymphoma L5178Y sublines*

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We have previously [1] reported differential cytotoxic and mutagenic effects of H₂O₂ treatment of L5178Y (LY) murine lymphoma sublines (LY-R and LY-S) at 4°C or 37°C. The closely related LY sublines are inversely cross-sensitive to ionizing radiation and hydrogen peroxide [2]. LY-S cells show a reduced ability to repair the X-ray induced DNA double strand breaks (dsb) as compared to LY-R cells [3]. Continuing studies on the response of these LY murine lymphoma sublines to H₂O₂ treatment we have now examined the initial DNA damage induced under the same conditions.

Genotoxicity of H₂O₂ is due to the product of the Haber-Weiss reaction (also called the Fenton type reaction) cycle (proceeding in the presence of transition metal ions), the highly reactive hydroxyl radical (OH•) [4]. OH• can react with DNA and generate single (ssb) [4-6] and double (dsb) [7] strand breaks and thymine glycol residues [8]. It is reasonable to expect that the amount of initial DNA damage is an essential factor defining the cytotoxic and genotoxic effects of H₂O₂ treatment. This report has been aimed at answering the question whether there is a relation between the level of DNA damage induced by hydrogen peroxide and its cytotoxicity in LY cells.

DNA damage was estimated by the "comet" assay, a rapid, simple and sensitive method for detecting DNA strand breaks in mammalian cells [9, 10]. The damaged cells are embedded

in an agarose sandwich on a microscope slide, lysed, placed in alkali, electrophoresed and stained with ethidium bromide (EtBr) (cf. Fig. 1). The lysing solution removes most of the cell contents, leaving the nuclear DNA with a small amount of associated protein. If the DNA contains strand breaks, unwinding occurs in alkali, allowing relaxed coils of DNA to be drawn from the nucleus during electrophoresis. Undamaged DNA remains within the nucleus and its amount is estimated from EtBr fluorescence.

Figure 1 presents the initial DNA damage in LY-R and LY-S cells exposed to hydrogen peroxide. In either line there was significantly less damage at 4°C than at 37°C both at 5 and 10 μM H₂O₂; however, the difference between LY-R and LY-S cells in the response to H₂O₂ at either temperatures tested was not statistically significant.

This observation is consistent with the assumption that DNA damage and, hence, H₂O₂ toxicity are caused by OH radicals generated at the sites containing reduced metal ions (Cu¹⁺, Fe²⁺), whereas the effect of temperature depends on availability of the ions. At temperatures that inhibit cellular metabolism, chromatin-bound transition metal ions do not undergo repeated reduction ("recycling effect"), because the reducing equivalents are not produced. Oxidized metal ions, mostly Fe³⁺, do not react with H₂O₂ [5, 11, 12]. Other factors that may have a protective effect against H₂O₂

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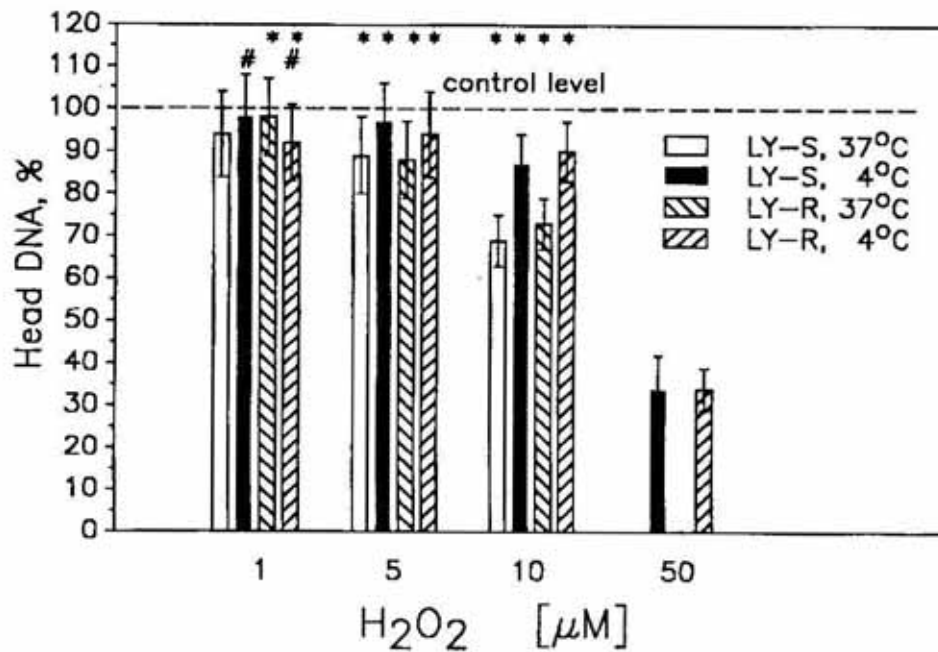


Fig. 1. Initial damage of DNA induced by H₂O₂ at 4°C or 37°C in LY sublines.

Cells (2×10^5) were treated with H₂O₂ in phosphate-buffered saline, pH 7.2 at 4°C or 37°C, then embedded in agarose, lysed in "high salt" buffer (pH 10) and electrophoresed (24 min, 20 V) [9, 10]. "Comets" were stained with ethidium bromide and intensity of fluorescence was measured using a computer aided microscope image analysis system; 50 comets were scored per experimental point. DNA damage was expressed as percentage of DNA remaining in the head of comet (corresponding to cell nucleus) after electrophoresis. Significance of difference of variances was estimated by one-way ANOVA, then significance of difference of means between samples was estimated by Student's *t* test.

*, Significant difference between cells of the same subline treated at different temperatures $P < 0.01$; #, significant difference between cells of the two sublines treated at same temperature $P < 0.01$.

action are "protective enzymes", i.e. glutathione peroxidase (GP), superoxide dismutase (SOD) and catalase (CAT). However, LY sublines did not differ in GP activity, the activity of SOD was higher in the LY-R cells, whereas LY-S cells had higher CAT activity [13]. Judging from the initial DNA damage it seems that, in LY cells, neither SOD nor CAT activity diminished the oxidative damage to the nucleus.

From the results reported above it follows that the initial damage measured by the "comet" method and expressed as the "head" DNA is not the critical factor that defines the difference in sensitivity of LY sublines to H₂O₂. A difference in the quality or location of lesions, however, cannot be excluded and should be examined with the use of other methods.

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