HYDROGEN PEROXIDE INDUCES DNA SINGLE STRAND BREAKS IN RESPIRATORY EPITHELIAL CELLS

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Abstract—The respiratory epithelium is often exposed to oxidant gases, including ozone from photochemical smog and toxic oxygen metabolites released from neutrophils recruited in conditions of airway inflammation. We evaluated DNA single strand break formation by alkaline elution as a measure of oxidant-induced DNA damage to bronchial epithelial cells. Human AdenoSV-40-transformed bronchial epithelial cells (BEAS), subclone R1.4 or nonhuman primate bronchial epithelial cells were cultured in growth factor supplemented Ham's F12 medium on polycarbonate filters. DNA was labeled by incubation with [3H]thymidine. Cells were incubated for 1 h in HBSS or HBSS and increasing concentrations of hydrogen peroxide (H$_2$O$_2$). Cells incubated in H$_2$O$_2$ demonstrated dose-dependent increases in strand break formation, and BEAS cells were more sensitive to H$_2$O$_2$-induced injury than primary bronchial epithelial cells. The addition of catalase or preincubation of cells with the iron chelator desferoxamine prevented H$_2$O$_2$-induced strand breakage. DNA strand break formation may be an important mechanism of oxidant injury in respiratory epithelial cells.

INTRODUCTION

The respiratory epithelium is often exposed to oxidant gases, including ozone from photochemical smog and toxic oxygen metabolites released from neutrophils recruited in conditions of airway inflammation. Damage to DNA has been shown in response to oxidant exposure; however, a wide range of differences

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exist among mammalian cells in sensitivity to DNA damage (1). Little is known about the direct sensitivity of the bronchial epithelium to oxidant-induced DNA damage or the mechanisms of injury following oxidant exposure.

Injury to DNA from oxidants has been studied extensively in bacterial species such as *E. coli*. DNA single strand breaks occur in bacteria following exposure to H$_2$O$_2$, O$_2^-$, and gamma radiation (2). *E. coli* defective in enzymes responsible for recombinational repair are more sensitive to H$_2$O$_2$-induced injury (3–5). Furthermore, H$_2$O$_2$-induced injury in *E. coli* appears to be mediated by a Fenton reaction, as bacterial killing is inhibited by metal chelators (6).

Oxidant-induced damage to DNA in mammalian cells appears to be more complex. Exposure to oxidants such as H$_2$O$_2$ may cause damage to DNA, including DNA strand breakage or base modification. While H$_2$O$_2$ easily enters cells, it does not interact with DNA directly, and it appears that H$_2$O$_2$-derived hydroxyl radical (OH·) or another hydroxyl-derived product reacts with cellular DNA to produce DNA damage. Alternatively, oxidant exposure may increase intracellular calcium concentrations, resulting in activation of endonucleases and subsequent DNA fragmentation (7, 8).

DNA strand break formation has been studied in bovine pulmonary artery and aortic endothelial cells. Using ethidium bromide as a marker of DNA unwinding and strand breakage, incubation of either cell type with as little as 50 μM H$_2$O$_2$ for 30 min resulted in significant DNA strand breaks (9). No strand breaks were detected when cells were incubated in lower concentrations of H$_2$O$_2$, and no difference in strand break in response to H$_2$O$_2$ was detected between pulmonary artery and aortic endothelial cells. DNA strand breaks also occurred in these cells when exposed to oxidants generated by xanthine and xanthine oxidase. Strand breaks were prevented by catalase, but not by hydroxyl radical scavengers (9).

DNA single strand break formation can be measured by alkaline elution of the DNA (10). We wondered if measurement of DNA single strand breaks might provide a sensitive method to detect injury to cells of the respiratory epithelium, and provide insights into the mechanisms responsible for oxidant induced events in the airway.

MATERIALS AND METHODS

**Bronchial Epithelial Cell Culture.** Human bronchial epithelial cells from the adenovirus type 12, SV-40 hybrid transformed BEAS-2B R1.4 subclone were stored frozen in liquid nitrogen until ready for use. Cells were plated onto collagen-coated 2 μm polycarbonate filters (Nuclepore Corp, Pleasanton, California) five to seven days prior to experiments and grown in Ham's F12 (Gibco) growth factor-supplemented medium at 37°C in a humidified atmosphere of 5% CO$_2$ and 95% air. F12 was supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), epidermal growth factor (25