Discrimination of Cigarette Smoke-Induced Stress Pathways by Inhibitors of the Fenton Reaction

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Introduction

DNA strand breaks as well as stress gene expression, *e.g.*, heme oxygenase (HO), are induced by cigarette smoke (CS) as oxidative stress-related effects in cellular systems *in vitro*. Hydroxyl radicals generated by the Fenton reaction have been shown to be involved in CS-dependent DNA strand breaks (1) but are also known as stress gene inducers (2). Using specific inhibitors of the Fenton reaction, the objective of this study was to evaluate

- a detailed mechanism for CS-dependent DNA damage, and
- whether CS-induced stress gene expression is also caused by hydroxyl radicals.

Methods

Smoke-bubbled PBS was prepared by bubbling 30 puffs of mainstream smoke generated from the University of Kentucky standard reference cigarette 2R1 through 10 ml PBS-Dulbecco. Swiss 3T3 fibroblasts or V79 hamster embryo lung cells were kept under standard conditions and exposed to smoke-bubbled PBS in the absence of serum. DNA strand breaks were evaluated using the alkaline elution technique (3). HO gene expression was monitored following standard Northern blotting protocols.

Results and Discussion

1. CS-Dependent DNA Damage is Related to Reactive Oxygen Species Produced by the Fenton Reaction

Exposure of eukaryotic cells to smoke-bubbled PBS for 1 hour leads to a concentration-dependent increase in DNA strand breaks (Figure 1).

The results described in Table 1 together with published data (1) indicate that the genotoxic activity of CS trapped in aqueous solution is mainly based on the formation of hydroxyl radicals - produced by the Fenton reaction:

$$O_2^{\cdot-} + H_2O_2 \xrightarrow{Fe^{2+}/Fe^{3+}} O_2 + OH^- + OH^{\cdot} \quad \text{(Fenton reaction)}$$

According to these data, the following scenario for the formation of DNA strand breaks in smoke-bubbled PBS-exposed cells is suggested (Figure 2):

Figure 1:
Alkaline DNA Elution of V79 Cells Exposed to Increasing Doses of Smoke-Bubbled PBS

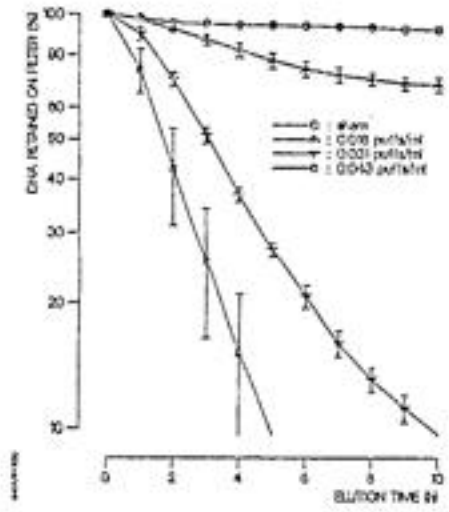
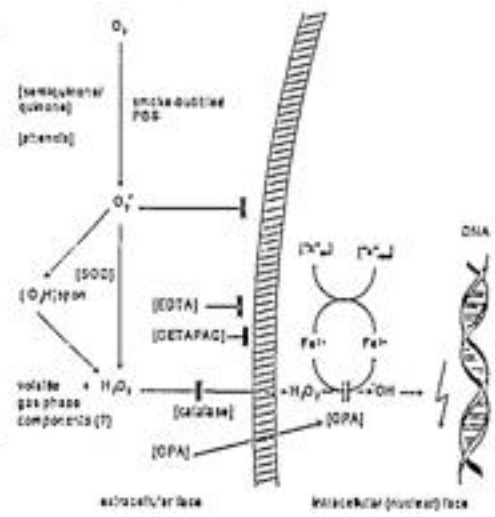


Table 1:
Effects of Scavenger on DNA Strand Breaks Caused by Smoke-Bubbled PBS (0.03 p/ml) in a Cell or Cell-Free Assay System

Treatment	Target	Assay		% Inhibition of DNA Strand Breaks
		Cells (V79)	Cell-Free (Naked DNA)	
SOD (900 U/ml)	$O_2^{\cdot-}$	+	-	0
catalase (40 U/ml)	H_2O_2	+	-	60.0
		-	+	91.0
o-phenanthroline (OPA) (0.1 mM)	$Fe^{2+/3+}$	+	-	95.5
EDTA (20 mM)	Fe^{2+}	+	-	0
		-	+	78.4
DETAPAC (1 mM)	Fe^{3+}	-	+	63.5
Na-Benzoate (10 mM)	$^{\cdot}OH$	-	+	100

Figure 2:
Proposed Reaction Sequence Leading to the Formation of CS-Dependent DNA Strand Breaks



2. CS-Dependent Stress Gene Response is *Not* Related to Reactive Oxygen Species Produced by the Fenton Reaction

Aqueous fractions of CS induce HO expression in exposed 3T3 cells (4):

Hydroxyl radicals generated by the Fenton reaction have been shown to induce stress genes including HO (2). However, neither catalase nor OPA exhibit any inhibitory effect on HO expression in smoke-bubbled PBS-treated 3T3 fibroblasts (Figure 4), although both scavengers are also potent inhibitors of CS-dependent DNA strand breaks in these cells (Figure 5).

Figure 3:
Kinetics of HO Expression in 3T3 Cells Exposed to 0.03 p/ml Smoke-Bubbled PBS

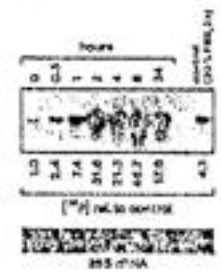
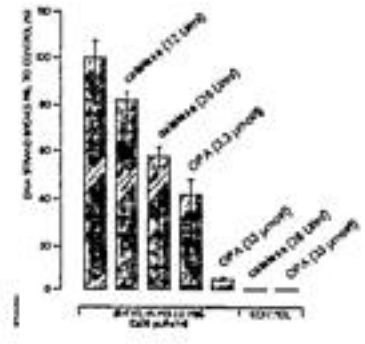


Figure 4:
Neither Catalase (A) nor OPA (B) Inhibit HO Expression in 3T3 Cells Exposed to 0.03 p/ml Smoke-Bubbled PBS

Figure 5:
Catalase and OPA are Potent Inhibitors of CS-Dependent DNA Strand Breaks in 3T3 Cells



Conclusions

DNA strand breaks induced by CS are mainly attributable to reactive oxygen species generated by the Fenton reaction. In contrast, CS-induced stress gene response is not affected by inhibitors of the Fenton reaction. Therefore, it is concluded that CS triggers at least two stress pathways *in vitro*, one Fenton-related and another non-Fenton-related.

References

(1) Nakayama *et al.*, 1985, *Nature*, **314**, 462 (2) Keyse and Tyrrell, 1989, *PNAS*, **6**, 99
(3) Kohn *et al.* in: Friedberg and Hanawalt (Eds.): DNA repair: A laboratory manual of research procedures, New York: Marcel Dekker, 1981, p. 379
(4) Müller and Gebel, 1994, *Carcinogenesis* **15**, 67