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DNA Fragmentation and DNA Repair of Mammalian Cells as an Indicator for the Complex Interactions Between Carcinogens and Modulating Factors*

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I. Introduction

IN THIS age of advanced technology, chemical compounds are playing an increasingly important role in our environment in the form of food additives, pharmaceutics, cosmetic products, and industrial reagents. With society becoming more conscious of the potential health hazard posed by some of these chemical agents, considerable effort has been devoted to identification of carcinogenic and mutagenic capacities of chemical compounds. Less attention has been focused on analysis of complex interactions among carcinogens, cocarcinogens, and inhibiting factors. Our understanding of biological effects of chemical agents is sufficient to unravel many of the reactions that occur when a multitude of factors are involved.

In this paper, we present a few examples of complex interactions among chemical carcinogens, DNA repair, and modulating factors of carcinogen action.

II. Interactions between Carcinogens and DNA

It is widely recognized that chemical carcinogens interact with DNA. By virtue of their chemical structure, direct-acting agents have the inherent property of reacting with DNA without undergoing further structural change. Precarcinogens require metabolic activation before they can bind to DNA. In both instances, the reactive forms are electrophilic species that attack DNA at specific sites in nucleotide bases or phosphodiester bonds (44); DNA strand breakages or structural distortions are produced.

III. DNA Repair Mechanisms

Photoreactivation. Repair of DNA damage by photoreactivating enzymes has been demonstrated in both microbial systems (37) and mammalian cells (27). In this process, direct reversal of altered DNA structure to the original functional form occurs upon exposure to visible light.

Excision Repair. In this repair process, nucleotides are removed in the vicinity of the DNA lesions, followed by resynthesis and rejoining of the DNA (36, 37). Although excision repair is extensively studied and its molecular basis is fairly well understood, the precise role of this apparently "error free" repair mechanism in the survival, mutagenesis, and carcinogenesis of mammalian cells remains unknown.

Replication and Postreplication (Recombination) Repair. These two types of repair have attracted great attention in recent years. However, current biochemical techniques do not permit an absolute dis-

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tinction between the two mechanisms. DNA lesions repaired by the replication pathway are believed to take place during semiconservative replication. In postreplication repair, sister strand exchanges between two replicating DNA molecules containing pyrimidine dimers or other alterations could restore a normal base sequence (31).

Other Repair Pathways. Several repair pathways have been identified in eukaryotic cells. For example, repair of cross-links in human cells following exposure to mitomycin C does not involve the excision pathway (10). Repair independent of pyrimidine dimers has also been observed after *gamma* or UV irradiation (6, 11).

Carcinogen-Induced DNA Repair. DNA repair synthesis occurs in cultured mammalian cells following exposure to a great array of chemical carcinogens (32, 43, 48, 49). Within a group of structurally related agents, the level of DNA repair observed is correlated with the carcinogenic potential of the test compound (41).

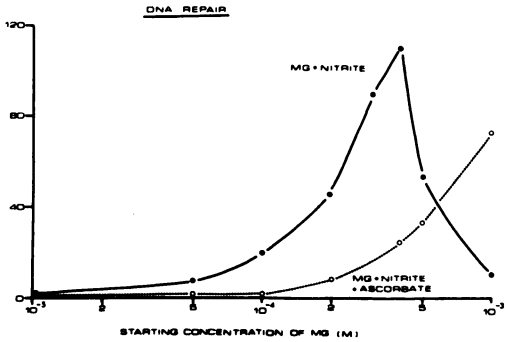

FIG. 1. Unscheduled incorporation of ^{3}HTdR into cultured human fibroblasts after exposure to methylguanidine, nitrite and ascorbate. ●—●, nitrosation in the absence of NaAsc; O····O, nitrosation in the presence of NaAsc (MG:$NaNO_2$:NaAsc was 1:3:1.5 molar ratio).

Recent results indicate that repair after treatment with some carcinogens (e.g., polycyclic aromatic hydrocarbons, alkylating agents) shares some common features with repair of UV- and/or *gamma*-ray damage (1, 21, 22, 28). Repair after treatment with carcinogens like aflatoxin B_1 (34), β-propiolactone (9, 23), N-methyl-N'-nitro-N-nitrosoguanidine (25, 26), and nitrosamine derivatives (8) involves processes that are not well understood.

IV. Modulating Factors of Carcinogen-DNA Interactions

The past emphasis on the interaction of chemical carcinogens with DNA has somewhat detracted from the numerous factors that have a modulating influence on the extent of DNA fragmentation and repair and thus on cell sensitivity. The necessity of metabolic activation of precarcinogens to electrophilic molecules has been generally accepted (24). There are many factors that are less well understood and appreciated, although they may have a pronounced influence on DNA repair. A short review of these modulating factors reveals their importance.

V. Modulating Effect of Noncarcinogenic Chemical Agents on Carcinogen Action

In view of recent advances in our understanding of the metabolic reactions of chemical carcinogens, the control of carcinogenesis may be effected through increas-

TABLE 1

Unscheduled incorporation of [^{3}H]TdR (grains per nucleus) into cultured human cells exposed to DMN, the S-9 activation mixture, and different reducing agents

Concentration of Reducing Agent (M)	Dimethylnitrosamine Plus S-9 Plus Reducing Agent			
	Ascorbate	Cysteine	Cysteamine	Propyl gallate
1×10^{-2}	3.9	9.7	4.4	2.8
3×10^{-3}	6.5	14.4	9.6	4.7
1×10^{-3}	7.9	15.7	10.9	7.8
3×10^{-4}	23.7	25.7	22.6	16.4
DMN without reducing agent	23.0	26.1	24.6	27.2

ing biodegradation or inhibiting metabolic activation of carcinogens. Alternatively, since the ultimate carcinogenic forms of most, if not all, chemical carcinogens are strong electrophilic reactants (24) or free radicals (35), interaction of these reactive species with DNA can be inhibited or prevented by blocking their formation or by trapping radicals. By monitoring DNA repair synthesis in cultured human fibroblasts, the in vitro inhibition of carcinogens can be demonstrated with the concomitant administration of reducing agents. For example, the nitrosation of methylguanidine can be prevented by sodium ascorbate (fig. 1). Reactive species from activated dimethylnitrosamine can be trapped by addition of reducing agents (table 1). Similarly, the mutagenic and DNA-damaging capacity of a direct-acting agent can be prevented by a trapping agent (table 2). That this phenomenon results from the electron scavenging effect of reducing agents is shown by the unimpaired action of UV (table 2).

VI. Inhibitory Action of Noncarcinogenic Chemical Agents on DNA Repair

Temporary inhibition of carcinogen-induced DNA repair constitutes one aspect of the modulation of carcinogen action. There is ample evidence showing that a genetically impaired DNA repair mechanism increases the sensitivity of bacterial and mammalian cells to the lethal and mutagenic effect of physical and chemical agents (15, 20–22, 33, 40, 42). Using DNA fragmentation and repair assays in cultured mammalian cells, it is possible to demonstrate inhibition of DNA repair by noncar-

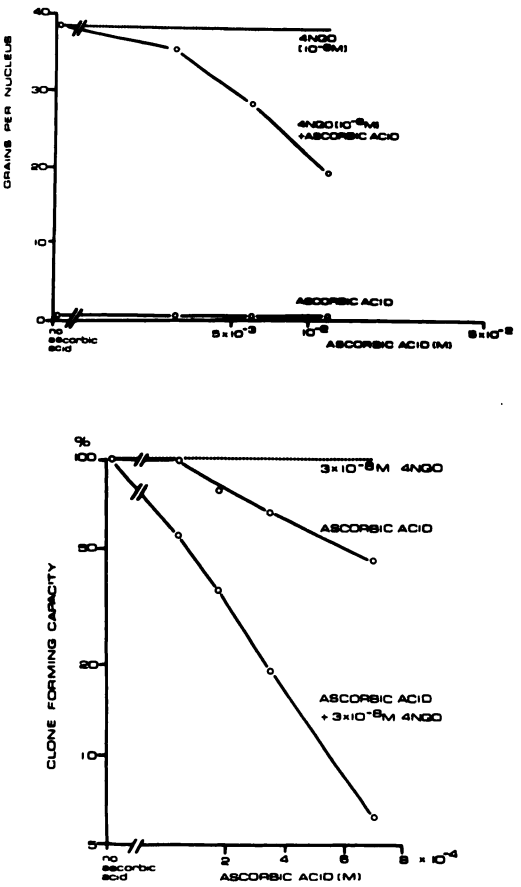

FIG. 2. Ascorbic acid-induced sensitization of cultured human fibroblasts to the carcinogen, 4-nitroquinoline 1-oxide, measured as a reduction in clone-forming capacity or as inhibition of DNA repair.

TABLE 2

Effect of cysteine on mutagenic action and DNA-damaging capacity of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)

	Chinese Hamster Ovary Cells		Salmonella	
	DNA repair (grains/nucleus)	Colony forming efficiency (%)	Mutagenic activity (His^+ revertants/10^7 survivors)	Survival (%)
MNNG*	22†	17.6‡	69.7	83
MNNG and cysteine	2†	31.1‡	0.3	101
Cysteine*	2	95.9‡	0	100
UV*	23	N.T.	N.T.	N.T.
UV and cysteine	28	N.T.	N.T.	N.T.

* MNNG, 1×10^{-5} M; cysteine, 2.5×10^{-3} M; UV, 80 ergs/mm², unless otherwise indicated; N.T. = not tested.

† MNNG, 2.5×10^{-4} M.

‡ MNNG, 2×10^{-5} M; cysteine, 1×10^{-4} M.

cinogenic chemical agents. Although many, if not all, of these compounds also inhibit regular DNA replication, and thus cannot be considered specific "DNA Repair Inhibitors" (7), they convey an increased sensitivity to cells treated with carcinogens and mutagens (38). Ascorbic acid, for example, seems to belong to this group (fig. 2).

VII. Complex Patterns of Carcinogen Action

Extensive efforts have been devoted to uncovering the mutagenic action of synthetic compounds introduced accidentally or intentionally into the environment. Somewhat less attention has been given to the potential genetic hazard of chemicals that are an essential part of human nutrition or are formed within mammalian tissues and organs. Ascorbic acid belongs to this group of "neglected" naturally occurring compounds, although its capacity to form radicals and its interaction with viral (4) and bacterial (19) DNA should have raised suspicion about its possible mutagenic capacity. Considerable evidence shows that oxidation products of ascorbic acid are mutagenic for microbial and mammalian cells (table 3). The precise molecular nature of the ultimate mutagenic metabolite of ascorbic acid is unknown. It is difficult to assess at present whether hydroxyl radicals generated in the interaction of Cu^{++} with H_2O_2 are responsible for the observed DNA fragmentation and the genetic changes. The H_2O_2 arises from the oxidation-reduction activity of the ascorbic acid-dehydroascorbic acid system (50).

The catalytic activity of Cu^{++} on thiol compounds (cysteamine, cysteine, and glutathione) exemplifies how metals acting on antioxidants can generate DNA and chromosome-damaging species (table 4). While Cu^{++} ions or the thiol compounds alone did not induce any unscheduled DNA synthesis or chromosome aberrations, a combination of the two resulted in DNA and chromosome damage.

TABLE 3

Mutagenic action and DNA-damaging capacity of oxidation products of ascorbic acid

	Cultured Human Fibroblasts			Salmonella
	DNA repair (grains/nucleus)	% Metaphases with chromosome aberrations		His$^+$ revertants/10^6 survivors
Ascorbic acid	2	0	~15	2
Ascorbic acid and Cu^{++}	36	—*	26.5	160
Ascorbic acid and O_2	N.T.	28.5	15.0	N.T.
Ascorbic acid concentration	8×10^{-3} M	1×10^{-3} M	3×10^{-4} M	3.5×10^{-2} M

* Mitotic inhibition; N.T. = not tested.

TABLE 4

The catalytic activity of Cu^{++} ions on thiol compounds in generating DNA- and chromosome-damaging metabolites

Metal	Antioxidant	U.D.S.* (Grains/Nucleus)	% Metaphase Plates with Chromosome Aberrations†
Cu^{++} (10^{-5} M)	Cysteamine (10^{-3} M)	83	29.0
	Cysteamine (10^{-3} M)	0	9.7
Cu^{++} (10^{-4} M)	Cysteine (10^{-3} M)	52	24.6
	Cysteine (10^{-3} M)	0	0
Cu^{++} (10^{-4} M)	Glutathione (3×10^{-3} M)	28	12.8
	Glutathione (3×10^{-3} M)	0	0
Cu^{++} (10^{-4} M)	—	0	0

* Unscheduled DNA synthesis in cultured human skin fibroblasts following 3 hr exposure to reaction mixture.

† Unscheduled DNA synthesis in Chinese hamster ovary cells following 3 hr exposure to reaction mixture.

VIII. Modulating Effect of Hyperthermia on DNA Repair

Hyperthermia has returned recently to a position of interest in light of its possibilities as an agent enhancing the lethal action of chemotherapeutic agents and ionizing radiation. Hyperthermia also seems to enhance the action of chemical carcinogens (5, 12, 13), although the precise mechanism is still unknown. Host-cell reactivation of UV-irradiated adenovirus type 12 is temperature-sensitive (16), and it has been proposed that short-term exposure to hyperthermia results in cell survival curves that indicate repair of sublethal lesions (3, 5) and possible impairment of DNA repair (2). Studies on cultured human fibroblasts indicate that 1) the level of excision-repair synthesis is normal after the application of the carcinogen and mutagen 4-nitroquinoline 1-oxide (4 NQO) and hyperthermia (fig. 3), and 2) the shift of small molecular weight DNA molecules to control size is delayed by at least the time that cells are maintained at the elevated temperature (fig. 4). The results suggest that hyperthermia can affect the mutagenic/carcinogenic action of chemicals by altering the rate of repair of DNA damage, and the effect manifests itself in the stage of DNA repair fol-

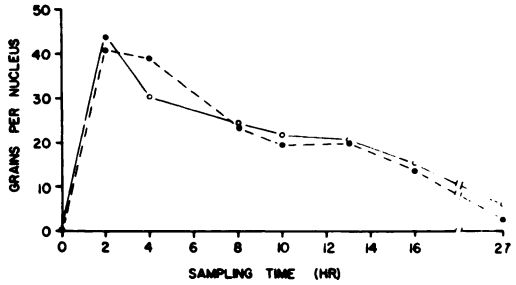

FIG. 3. Autoradiographic analysis of the effect of hyperthermic temperature on DNA repair in cultured human fibroblasts following 1.5 hr exposure to 4-nitroquinoline 1-oxide. Cells were placed into 37°C (●—●) or 41.8°C (○—○) for 8-hr posttreatment, and then returned to 37°C for the duration of the experiment. Each point represents the average number of grains in 50 to 100 counted nuclei following 2-hr labeling with ^{3}HTdR (10 μci/ml).

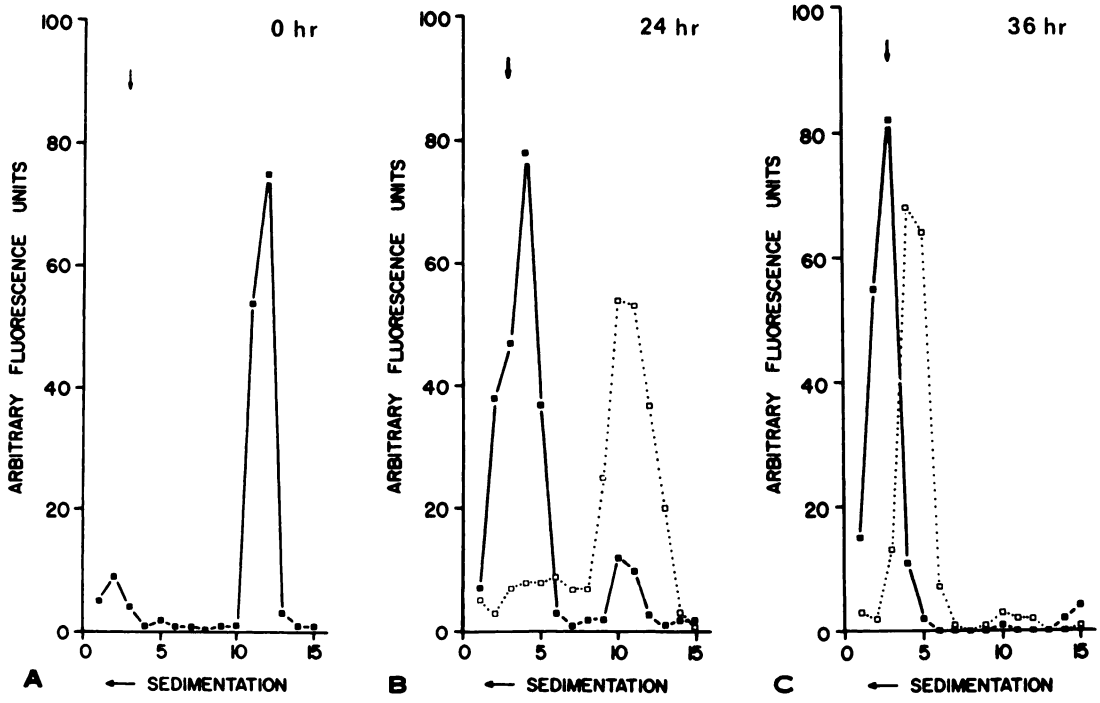

FIG. 4. Alkaline sucrose gradient sedimentation profile of cultured human fibroblasts following exposure to 1×10^{-6} M 4-nitroquinoline 1-oxide for 1.5 hr (A), subjected to 37°C (■—■) or 41.8°C (□····□) for 8-hr posttreatment and then returned to 37°C for a further 24 hr (B), or 36 hr (C).

TABLE 5
Applications of DNA fragmentation and repair assays

1. Induction of DNA damage and repair with and without metabolic activation.
2. Identification of photosensitizing agents (39).
3. In vitro simulation of formation of potent carcinogenic compounds [e.g., nitrosation (17)].
4. Modulating effect of noncarcinogenic agents on carcinogen action.
5. Estimation of variations within the human population in the sensitivity to chemical carcinogens.

lowing excision and resynthesis of damaged strands.

IX. Overview

This brief presentation has outlined how seemingly harmless chemical agents can become potential hazards and how repair of DNA damage is affected by chemical, as well as physical, modulating factors. Extreme hypoxic conditions (14) and high doses of carcinogens (32) suppress the DNA repair system. This temporary inhibition of repair may increase susceptibility to subsequent damage during exposure to carcinogens (45, 46).

The possibility of simulating complex conditions actually found in man is one of the features that makes the DNA fragmentation and repair assay in cultured mammalian cells an attractive in vitro system for studying carcinogen-DNA interactions (table 5). An understanding of the mechanism of interactions between carcinogens and modulating factors is essential for the design of preventive measures against chemical carcinogens. The suppression of the mutagenic (18, 29, 30) and oncogenic activity (47) of carcinogens by antioxidants indicates such a possibility in cancer prevention.

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