Oxidative Stress by Environmental Agents

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Abstract

A broad variety of environmental agents may exert their harmful effects through generation of oxidative stress. Failure of the system of enzymatic, endogenous and nutritional antioxidants may lead to mutagenic oxidative DNA damage as well as dysregulation of signaling and cell cycle control, resulting in carcinogenesis. Many environmental factors, including radiation, various kinds of air pollution, transition metal exposures and occupational exposures of many kinds have been shown to increase oxidative DNA damage in animal as well as human biomarker studies.

DNA repair enzymes, such as glycosylases, including *OGG1* and also nucleotide excision repair enzymes, including *ERCC1*, are highly active in order to prevent oxidative damage from resulting in mutations. Indeed, knockout animals accumulate relevant lesions. In a number of experimental models and human interventions we have studied the level of oxidative DNA damage and the expression of mRNA of *OGG1* and *ERCC1* by real-time RT-PCR and compared with the expression of a well known oxidative stress response gene, heme oxygenase 1 (HO1).

Ionizing radiation can induce oxidative DNA damage and HO1, whereas expression of DNA repair enzymes is only modestly affected. Experimental exposure to diesel particles by inhalation or the oral route can enhance *OGG1* and *ERCC1* mRNA expression in target organs with decreased or unchanged 8-oxodG levels or increased levels of strand breaks. The cooked food mutagen IQ had no effect on expression of *OGG1* and *ERCC1* or 8-oxodG level, despite increased DNA strand breaks and bulky adduct levels in colon and liver. After partial hepatectomy *OGG1* expression increased substantially without a correspondingly increased incision activity in rats, although *OGG1* expression and incision activity were concomitantly increased in foetal rats compared to adult rats.

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In human biomonitoring studies seasonal variation of oxidative DNA damage, including strand breaks and 8-oxodG in lymphocytes are apparent with increased values possibly related to solar exposure. Moreover, over 5 months the mRNA levels of *OGG1* and *ERCC1* in white blood cells showed seasonal variation parallel to the level of solar radiation, suggesting a regulatory mechanism. Even with modest levels of urban air pollution significant correlations between personal exposure and biomarkers of oxidative stress in plasma and 8-oxodG in lymphocytes have been shown in a biomonitoring study.

The aggregated data indicate that DNA repair enzymes, including *OGG1*, are subject to substantial regulation and that oxidative stress may be an inducer. However, the relationship between mRNA levels and incision activity is not simple and levels of oxidative DNA damage in cells must be interpreted in the light of the repair capacity in particular when studying environmental factors. Exposure to urban air pollution, even at modest levels, appears to be an important inducer of oxidative stress and DNA damage.

Key words: antioxidants, biomarkers, DNA repair, gene expression, oxidative DNA damage.

Introduction

Cells are constantly exposed to oxidants from metabolic and other biochemical reactions as well as external factors. Oxidative modifications of DNA are abundant, mutagenic and thought to be important in carcinogenesis and aging as supported by experimental studies in animals and *in vitro* [1−3]. Indeed cells are constantly exposed to oxidants from both physiological processes, such as mitochondrial respiration [4], and pathophysiological conditions such as inflammation, ischemia/reperfusion, foreign compound metabolism and radiation [1]. A broad variety of environmental agents may exert their harmful effects through generation of oxidative stress. Many environmental factors, including radiation, various kinds of air pollution, transition metal exposures and occupational exposures of many kinds have been shown to increase oxidative DNA damage in animal as well as human biomarker studies (Fig. 1). Damage to DNA may be important in exposure related carcinogenesis as the DNA base lesions such as 8-oxo-7, 8-dehydro-2'-deoxyguanosine (8-oxodG) are abundant and highly mutagenic [2].

The bodily defences against oxidants include an extensive system of antioxidant enzymes and radical scavengers and chain breakers, of which many are nutritionally dependent. Failure of the system of enzymatic, endogenous and nutritional antioxidants may lead to mutagenic oxidative DNA damage as well as dysregulation of cell cycle control, resulting in carcinogenesis. Fortunately, damage to DNA is also repaired with high efficiency in the cells in the body [5, 6]. DNA repair enzymes, such as glycosylases, including *OGG1* and also nucleotide excision repair enzymes, including *ERCC1*, appear highly active in order to prevent oxidative damage from resulting in mutations. However, little is known of regula-

tion of these genes in relation to oxidative stress and DNA damage.

Urban air pollution, in particular vehicle emission, is one of the most important environmental factors affecting health in modern cities. Air pollution may inflict health effects through several modes of actions. The carcinogenic effect of diesel emission particulate matter (DEPM) has generally been ascribed to DNA adducts from polyaromatic hydrocarbons (PAH) and other similar compounds [7]. However, DEPM contains, soot, metals and various PAHs and induces genotoxic effects not only in terms of PAH-DNA adducts but also oxidative DNA damage, which was correlated with mutagenicity and tumour formation in animal experiments [8, 9]. PM can induce oxidative stress mediated by a particle induced inflammation causing macrophages to release reactive oxygen species (ROS), by transition metals on the particle surface capable of generating ROS through the Fenton reaction or by quinones in the particles that produce ROS through redox cycling [10－12]. Several studies have demonstrated that diesel exhaust particles induce production of ROS both with [12, 13] and without [14, 15] the presence of biological activating systems. Several experimental studies have found increased 8-oxodG levels after exposure to PM both *in vitro* [16, 17] and *in vivo* [8, 9, 18, 19].

Indeed, removal of PAH has little effect on carcinogenesis and formation of oxidative DNA damage [9]. Urban air pollution also contains a number of direct oxidants, nitrogen oxides and ozone. Moreover, the aliphatic and aromatic compounds may induce generation of reactive oxygen species during their metabolism [20].

Air Pollution and Biomarkers of Oxidative DNA Damage

For molecular epidemiological studies oxidatively modified nucleobases can be measured by various chromatographic techniques in DNA isolated from e.g. lymphocytes, sperm cells or tissues. In DNA more than 100 different oxidative modifications have been observed [21, 22]. However, so far only a few of the base modifications have been used as biomarkers and of these the oxidative C-8 adduct of guanine is by far the most studied as either the nucleoside (8-oxodG) or base (8-oxoguanine) [2, 23]. In principle, the level in nuclear or mitochondrial DNA from target or surrogate tissues or cells or the excretion of repair products into the urine can be measured. Strand breaks can be detected in cells or tissue by the comet assay [24]. By the optional use of relatively specific repair enzymes, e.g. fapyguanine glycosylase (Fpg) and endonuclease III breaks can be introduced at oxidized bases allowing quantification. Oxidized bases and nucleosides from DNA repair processes, sanitation of the nucleotide pool and cell turnover can be measured in urine [23]. So far, mainly 8-oxodG, 8-oxoguanine, thymine glycol (Tg), dTg and 5-OH-methyluracil have been used in human urine [25, 26－28]. It should be emphasized that the urinary excretion rate represents the average rate of damage in the total body whereas the level of oxidized bases in DNA is a concentration measurement in that specific tissue/cells in the moment of sampling.

Table I. Oxidative DNA damage and exposure to polluted air in humans

Chemical exposure	Increased biomarkers	No increase
Tobacco smoking	8-oxodG in urine, WBC and sperm, comet SSB	comet assay 8-oxodG in WBC and sperm
Urban air pollution	8-oxodG and 8-oxoGua excretion 8oxodG in WBC, comet SSB	
Benzene	8-oxodG excretion comet SSB	8-oxodG in WBC comet SSB, Fpg
Styrene, solvents,	comet SSB	
Vinyl chloride	comet SSB	Fpg/Endonuclease III sensitive sites
Waste matter	comet SSB	
Wood dust	comet SSB	comet SSB

Adapted from [2, 23, 32, 33, 39, 66, 67], SSB: single strand breaks, Fpg: fapyguanine glycosylase

A number of studies in humans have shown associations between exposure to air pollution and oxidative damage to DNA (Table 1). We have recently shown that bus drivers from the center of Copenhagen exhibited signs of more oxidative DNA damage in terms of 8-oxodG excretion than drivers from the suburban and rural districts [29]. In addition, PAH adducts, chromosomal aberrations and oxidation of plasma proteins and lipid peroxidation were increased in the bus drivers from the center [30, 31]. Moreover, in a group of young subjects followed during the four seasons of a year we could show significant correlation between personal exposure to particulate matter in terms of $PM_{2.5}$ (mass of particles with a diameter less than 2.5 μm) and black smoke and the level of oxidative damage to DNA, protein and lipid in the blood [32, 33]. Short term exposure to traffic pollution in 3 subjects in Tokyo resulted in an increase in oxidative DNA damage estimated from excretion of 8-oxoguanine, a repair product of this lesion in DNA [34]. Similarly, the excretion of 8-oxodG was increased after occupational exposure to benzene and fumes from art glasswork as well as by tobacco smoking and environmental tobacco smoke [35, 36]. However, the effect of smoking on lymphocyte DNA damage has not been consistent (Table 1). The level of 8-oxodG was increased in DNA from central sites of the lung in smokers and was correlated with the level of carboneceous particles which could originate from DEPM [37, 38]. Associations between air pollution exposure, in particular to ozone and DNA strand breaks assessed by the comet assay on leukocytes and nasal epithelium have been shown in Mexico (reviewed in [39]).

In guinea pigs we have shown that intratracheal administration of diesel particles induced DNA damage assessed by 8-oxodG levels and strand breaks by the comet assay in the lungs [19]. Similarly, exposure to ozone by inhalation in mice induced inflammation and strand

breaks in bronchiolar lavage cells, whereas the level of 8-oxodG was not affected in the lung tissue [40]. Interestingly, oral exposure to diesel particles in the diet resulted in DNA damage in terms of strand breaks and PAH adducts, oxidative stress as well as apoptosis and upregulation of DNA repair enzymes in colon epithelial cells and liver in rats. These effects were seen at a relatively modest exposure level, which could easily be encountered in an occupational setting.

Regulation and Biomarkers of DNA Repair Enzymes

Effects of environmental agents causing oxidative damage to DNA may be modified by altered regulation of DNA repair enzymes (Fig. 1). Thus, the results of such interventions may be difficult to interpret without assessment of DNA repair enzymes. Although definitely possible the use of protein levels and activity of such enzymes are technically difficult to apply to multiple samples of e.g. white blood cells from intervention studies. Accordingly, mRNA levels of the enzymes may be measured by means of e.g. real-time PCR with great precision, accuracy and capacity. Moreover, cDNA arrays may be used to screen ef-

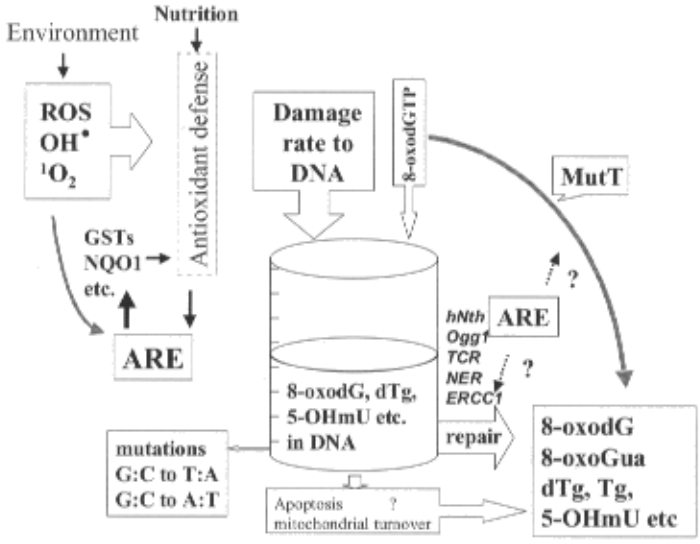

Fig. 1. Possible role of antioxidants and the antioxidant responsive element (ARE) for the mass balance of 8-oxodG formation in DNA and nucleotide pool and fates in terms of repair, cell and mitochondrial turnover and mutations. ROS are reactive oxygen species. GSTs and NQO1 are glutathione S-transferases and NAD(P)H: quinone reductase, respectively. MutT includes 8-oxodGTP phosphatase and 8-oxodGMP nucleotidase sanitizing the nucleotide pool. Repair result in 8-oxodG or 8-oxoGua by transcription coupled repair (TCR) nucleotide excision (NER; *ERCC1*) and base excision (*OGG1* and *OGG2*).

Table 2. Evidence for regulation of OGG1 in terms of differential levels of mRNA, protein and/or activity in different species, organs and developmental stages

Tissue	mRNA (10^6/18S)	Influenced by	Reference
Lymphocytes human	10±6	seasonal variation	[64]
Testis rat	36±12		unpublished data
Fetal tissue rat	190±120	activity high	[47]
Lung mouse	9±5		unpublished data
Lung rats		increased by diesel particles	[51]
Colon rat	40±19		[68]
Liver rat	6±1	increased by:	
		hepatectomy (6 x increased)	[46]
		peroxisome prolif.	[48]
		phenobarbital	[49]
A549 cells		increased by asbestos	[50]
		mRNA+protein down by $Na_2(CrO_4)$	[52]

fects of batteries of such mRNAs. We are developing real-time PCR based measurement of mRNAs of important DNA repair enzymes, so far *OGG1* and *ERCC1* for use as biomarkers in human as well as experimental studies. Oxidations and alkylations of the nucleobases are typically repaired by base excision repair (BER), whereas bulky adducts are repaired by nucleotide excision repair (NER) [5, 6]. Both pathways are complex with many enzymes working in succession, and are probably intimately connected with overlapping substrate specificity.

OGG1 encodes the 8-oxoguanine-DNA glycosylase, which removes 8-oxoguanine from DNA as part of the base excision repair pathway [41]. *OGG1* overexpression has been shown to correlate with the repair capacity of 8-oxo-guanine [42]. Moreover, *OGG1* knockout animals accumulate 8-oxoguanine, in particular if subjected to oxidative stress [43−45]. Experimental studies show differential levels of mRNAs levels of *OGG1* in Table 2. Moreover, the levels of *OGG1* mRNA can be induced in various organs and cell culture systems after oxidative stress, and protein and or activity levels have been found to be enhanced

as well. After partial hepatectomy *OGG1* expression increased substantially without a correspondingly increased incision activity in rats [46], although *OGG1* expression and incision activity were concomitantly increased in foetal rats compared to adult rats [47]. Chemicals known to induce oxidative stress in the liver, partly through enhanced metabolism, including peroxisome proliferators and phenobarbital have been shown to enhance mRNA levels of *OGG1* [48, 49]. The effect of phenobarbital was maximal after 12 days and accompanied by normalization of earlier substantially increased levels of 8-oxoguanine [49]. A similar pattern was seen in cultured cells exposed to asbestos [50]. The mRNA level of *OGG1* has been shown to increase along with the activity after an initial decrease in rat lungs after instillation of diesel exhaust particles [51]. Downregulation of mRNA levels and the *OGG1* protein has also been shown in human alveolar epithelial cells exposed to cromate [52].

ERCC1 encodes a subunit of the endonuclease, which makes the incision 5' of the DNA damage in nucleotide excision repair [53]. Previous studies have shown that the mRNA levels of *ERCC1* correlates with the DNA repair capacity in various tissues [54−57]. Studies of *ERCC1* expression in cell lines indicate that *ERCC1* expression may be inducible [58, 59]. Interestingly, *ERCC1* also appears to be important for repair of 8-oxoguanine. Thus, *ERCC1* knockout mice have substantially increased levels of 8-oxoguanine in the liver, which is corrected after transgenic expression [60]. Moreover, the *OGG1* protein is not required for repair of oxoguanine in transcribed DNA sequences, where BRCA1 and BRCA2 seem to be important [61, 62].

The use of mRNA of *OGG1* as biomarker in humans has been suggested in a study of 5 subjects with repeated sampling [63]. In a human biomonitoring study running over 5 months we assessed the mRNA levels of *OGG1* and *ERCC1* in white blood cells by means of real-time RT PCR [64]. The levels of the two mRNAs showed a close correlation and a relatively constant level within an individual. Interestingly, there was a considerable seasonal variation with the highest values in late spring/early summer. The levels of *OGG1* and *ERCC1* mRNAs correlated significantly with the solar flux during 5 and 30 days prior to sampling, respectively, suggesting a regulatory mechanism induced by sunlight [64].

Conclusions

The aggregated data indicate that DNA repair enzymes, including *OGG1* and *ERCC1* are subject to substantial regulation and that oxidative stress may be an inducer, implying the use as biomarkers in antioxidant studies. It appears that upregulation in terms of several days *in vivo*, whereas the regulatory mechanism is unknown. It may involve mRNA stabilization as known to occur for oxidative stress induction of the important response gene heme oxygenase-1 [65]. However, the relationship between mRNA levels and incision activity is not simple and levels of oxidative DNA damage in cells must be interpreted in the light of the repair ca-

pacity.

Air pollution appears to be an important inducer of oxidative stress and DNA damage. Animal experimental studies are consistent in that aspect. In humans exposure to urban air pollution, even at modest levels, also appears to induce oxidative stress and DNA damage. In experimental animals DNA repair is also induced by air pollution exposure, whereas such effects have yet to be demonstrated in humans.

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