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Experimental Study of Oxidative DNA Damage

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Animal experiments allow the study of oxidative DNA damage in target organs and the elucidation of dose–response relationships of carcinogenic and other harmful chemicals and conditions as well as the study of interactions of several factors. So far the effects of more than 50 different chemical compounds have been studied in animal experiments mainly in rats and mice, and generally with measurement of 8-oxodG with HPLC-EC. A large number of well-known carcinogens induce 8-oxodG formation in liver and/or kidneys. Moreover several animal studies have shown a close relationship between induction of dative DNA damage and tumour formation.

In principle the level of oxidative DNA damage in an organ or cell may be studied by measurement of modified bases in extracted DNA by immunohistochemical visualisation, and from assays of strand breakage before and after treatment with repair enzymes. However, this level is a balance between the rates of damage and repair. Until the repair rates and capacity can be adequately assessed the rate of damage can only be estimated from the urinary excretion of repair products albeit only as an average of the entire body.

A number of model compounds have been used to induce oxidative DNA damage in experimental animals. The hepatocarcinogen 2-nitropropane induces up to 10-fold increases in 8-oxodG levels in rat liver DNA. The level of 8-oxodG is also increased in kidneys and bone marrow but not in the testis. By means of 2-nitropropane we have shown correspondence between the increases in 8-oxodG in target organs and the urinary excretion of 8-oxodG and between 8-oxodG formation and the comet assay in bone marrow as well potent preventive effects of extracts of Brussels sprouts. Others have shown similar effects of green tea extracts and its components. Drawbacks of the use of 2-nitropropane as a model for oxidative DNA damage relate particularly to formation of 8-aminoguanine derivatives that may interfere with HPLC-EC assays and have unknown consequences. Other model compounds for induction of oxidative DNA damage, such as ferric nitriloacetate, iron dextran, potassium bromate and paraquat, are less potent and/or more organ specific.

Inflammation and activation of an inflammatory response by phorbol esters or *E. coli* lipopolysaccharide (LPS) induce oxidative DNA damage in many target cells and enhance benzene-induced DNA damage in mouse bone marrow.

Experimental studies provide powerful tools to investigate agents inducing and preventing oxidative damage to DNA and its role in carcinogenesis. So far, most animal experiments have concerned 8-oxodG and determination of additional damaged bases should be employed. An ideal animal model for prevention of oxidative DNA damage has yet to he developed.

Keywords: Oxidative DNA damage, 8-oxodG, animal models, carcinogenesis, cancer prevention

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Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 2-NP, 2-nitropropane; GC/MS-SIM gas chromatography mass spectrometry-selective ion monitoring; HPLC-EC high performance liquid chromatography-electrochemical detection; EGCG, epigallocatechin gallate; TPA, 12-O-tetradecanoylphorbol-13-acetate; LPS, *E. coli* lipopolysaccharide MeIQx:(2-amino-3[8-dimethylimidazo-4,5-f]quionoxaline; DMBA, dimethylbenz[α]anthrazene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-I-butanone, N-ethyl-N-hydroxyethylnitrosamine

INTRODUCTION

Oxidative damage to DNA has been proposed to be an important factor in carcinogenesis supported by experimental studies in animals and *in vitro*.[1–3] Animal experiments allow the study of oxidative DNA damage in target organs and the elucidation of dose–response relationships of carcinogenic and other harmful chemicals and conditions as well as the study of interactions of several factors. So far, the effects of more than 50 different chemical compounds have been studied in animal experiments mainly in rats or mice and generally with measurement of 8-oxodG with HPLC-EC as recently reviewed.[4] A large number of well-known carcinogens induce 8-oxodG formation, in particular in liver and/or kidneys. Moreover, several animal studies have shown a close relationship between induction of oxidative DNA damage and tumour formation.

In principle the level of oxidative DNA damage in an organ or in cells may be studied by measurement of modified bases/deoxynucleosides in extracted DNA, by immunohistochemical visualisation, and from assays of strand breakage before and after treatment with repair enzymes.[5] However, this level is a balance between the rates of damage and repair. Until the repair rates and capacity can be adequately assessed the rate of damage can only be estimated from the urinary excretion of repair products, albeit only as an average of the entire body.[6,7] The major part of 8-oxodG in DNA arises from oxidation of the base within the DNA whereas incorporation of oxidised nucleotides from the cellular pool is probably of minor quantitative importance although highly mutagenic and thus of large qualitative importance.[8] The repair of 8-oxodG in DNA results in 8-oxodG or 8-oxoguanine by nucleotide excision and base excision, respectively.[9] Recently, the human 8-oxoguanine glycosylase (OGG1) was cloned by several groups,[10,11] whereas nucleotide excision repair was shown to contribute to the repair of 8-oxodG in DNA.[12] The third major potential source of urinary 8-oxodG relates to cell and mitochondria turnover.[2]

So far, very few of the animal studies have included oxidative DNA modifications other than 8-oxodG. A small number of studies have used GC/MS with selective ion monitoring for measurement of other oxidised bases in extracted and hydrolysed DNA.[13–17] However, the derivatisation procedure required for that assay may induce artifactual oxidation of the bases and the reported levels are indeed often higher than obtained by HPLC-EC.[14,16–19] Recent data concerning rat liver 8-oxodG obtained with HPLC-MS/MS give similar values as HPLC-EC.[20]

Particular interest relates to prevention of oxidative DNA damage. For that purpose models involving the induction of oxidative DNA damage by relevant compounds have been employed and a number of antioxidants, anticarcinogens, plant extracts and other compounds have been shown to have preventive effects. The present review will discuss such animal models for induction and prevention of oxidative DNA damage.

2-NITROPROPANE

The hepatocarcinogen 2-nitropropane is a very potent inducer of up to 10-fold increases in 8-oxodG levels in rat liver DNA.[21–28] Similar effects have been obtained with other secondary nitroalkanes.[29] After 2-NP administration the level of 8-oxodG is also increased about 2-fold in the kidneys and 5-fold in the bone marrow but

TABLE I The increase in 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in nuclear DNA from target tissues and 24-h urine after treatment with 2-nitropropane (2-NP, 100 mg/kg) and the estimated contributions to total body burden of guanine oxidation. Data are from Ref. [28]

Target	Representing part of body (%)	Relative increase	Contribution to total body burden (%)
Liver	3.5	8-fold	28
Kidney, bone marrow and other targets	3.0[a]	4-fold[b]	12
Urinary excretion	100	1.4-fold	40

[a] Estimated; [b] Estimated average.

not in the testis.[28] Moreover. the level of 8-oxodG in the bone marrow correlates closely with the comet assay in the same cells.[30] Similarly, we have shown that the temporary excesses in 8-oxodG levels in the target organs alter 2-nitropropane administration correspond reasonably to the increase in the urinary excretion of 8-oxodG (Table I).[28] This supports the view that 8-oxodG is an important repair product of 8-oxodG formation in the tissues and that the urinary excretion can be used as a biomarker in that respect.

The potent induction of 8-oxodG by 2-NP has been used in a number of studies of preventive effects of various compounds (Table II). We have recently shown that pretreatment with an extract of Brussels sprouts can abolish the increases in 8-oxodG in bone marrow and kidney and reduce the increases in liver and urine in rats treated by 2-NP 100 mg/kg.[28] Others have shown similar effects of green tea extracts, ellagic acid and vitamin E, whereas vitamin C and epigallocatechin gallate (ECGC) had no or minimal effects.[22,55] Benzyl selenocyanate can also reduce induction of 8-oxodG by 2-NP although the sulphur analogue benzyl thiocyanate had no effect.[25] Surprisingly, depletion of iron increased the 8-oxodG induction by 2-NP, whereas depletion of manganese and copper decreased the effect,[27] suggesting complicated roles of these transition metals.

Due to several problems, 2-NP is not an ideal model compound for the study of oxidative DNA damage. The major problem with 2-NP relates to the formation of 8-aminoguanine in DNA and

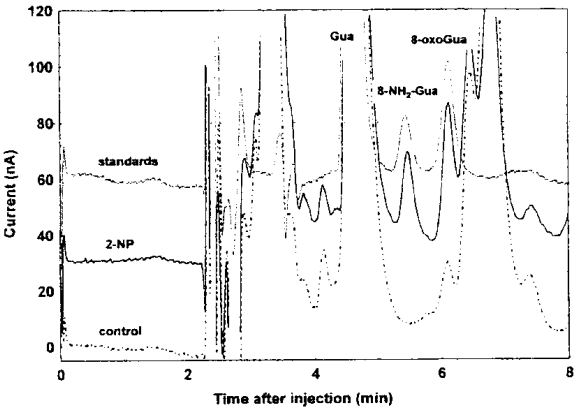

FIGURE 1 Chromatogram (electrochemical tracing) of HPLC analysis of 8-aminoguanine (8-NH_2-Gua) 8-oxo-7,8,-dihydroguanine (8-oxoGua) in nuclear DNA from rat liver collected 6 h after administration of 2-nitropropane (2-NP 100 mg/kg) or vehicle (control). The DNA was hydrolysed in formic acid at 130°C for 30 min and the chromatographic condition were as described elsewhere.[5] A chromatogram of a standard solution containing guanine (Gua) 250 μM, 8-NH_2-Gua 25 nM and 8-oxoGua 25 nM is also shown.

RNA.[23,56,57] The 8-aminoguanine derivatives are electrochemically active and behave similarly to the 8-oxoguanine derivatives in HPLC (Fig. 1). Thus, after formic acid hydrolysis of extracted DNA both base derivatives can be assayed by HPLC as described elsewhere.[5] In untreated rats no 8-aminoguanine derivatives could be detected, whereas 6 and 24 h after administration of 2-NP 100 mg/kg the levels were 9.0 ± 5.7 and 3.6 ± 1.4 per 10^5 intact guanine bases, respectively ($n = 3$–5/group; unpublished data). This indicates that the level of 8-aminoguanine is similar to the level of 8-oxodG induced by 2-NP and

they have the same repair/disappearance rate. Accordingly, 8-aminoguanine derivatives may interfere chromatographically with the corresponding 8-oxoguanine derivatives and the consequences of the aminated bases are not known, although they are likely to depurinate spontaneously.

Other problems with 2-NP as a model compound for induction of oxidative DNA damage relate to the requirement of metabolic activation, probably via sulphation.[56] Moreover, some cytochromes P450 appear to detoxify by denitrification. Depletion of these enzymes by cobalt protoporphyrin IX enhanced the effect, whereas induction by phenobarbital reduced the oxidative damage.[25] In rats there were substantial strain and sex differences in the effect of 2-NP on 8-oxodG formation[58,59] and in rabbits there was no oxidative DNA damage at all.[60]

TRANSITION METALS AND OTHER INORGANIC COMPOUNDS

Transition metals, e.g. iron catalyse the generation of hydroxyl radicals from hydrogen peroxide and could thus induce oxidative DNA damage.[61] However, several of the transition metals including copper and manganese as well as zinc, are essential for the function of superoxide dismutases and other important enzymes. Accordingly, overload with and depletion of metals may have complex effects on oxidative stress in experimental studies.

In isolated DNA the damaging effects of iron and copper and the prooxidant effects of reducing agents, such as vitamin C, are easily demonstrated.[62,63] Similarly, in isolated cells iron induce a range of oxidative modifications of the DNA bases.[64] However, *in vivo* iron appears to be much less potent. In rat testis the level of 8-oxodG was only 25% increased after administration of 500 mg/kg of iron-dextran,[65] whereas cotreatment with Arochlor 1254 was required for 600 mg/kg iron-dextran to increase the levels in mouse liver.[66] In our hands, 400 mg/kg of iron-dextran was required to raise the 8-oxodG levels in sperm cells and kidney tissue as well as its urinary excretion less than 2-fold, whereas no effects were seen in liver and testis, although iron was a very potent inducer of 8-oxodG in testis and sperm cells *in vitro* (unpublished data). On the other hand, ferric nitriloacetate effectively induced 8-oxodG levels and other DNA base modifications in rat kidneys and this has been employed for the study of preventive interventions as shown in Table II.[14,32,33,67–69] Surprisingly, depletion of iron increased the spontaneous levels and enhanced the inducing effect of 2-NP on 8-oxodG in rat liver,[27] although with a choline deficient diet iron depletion reduced the inducing effect on 8-oxodG.[50] Depletion of manganese and copper decreased both the spontaneous and 2-NP increased levels of 8-oxodG in rat liver.[27] These data suggest more complex roles of these transition metals, consistent with their involvement in both generation of oxygen radicals and antioxidant defence enzymes.

Cobalt and nickel have been shown to induce a range of oxidative DNA modifications in rats, particularly in the kidneys,[13,16] Cadmium chloride caused increased levels of 8-oxodG in the testis in rats although this could at least partly be related to a decrease in repair activity of this lesion.[70] Nevertheless *in vitro* strand breaks were induced by cadmium in isolated Leydig cells in keeping with rat testis as a target for the carcinogenic effect of cadmium.[71]

Depletion of zinc may cause oxidative damage. Thus, maternal depletion resulted in increased 8-oxodG levels in the livers of infant rhesus monkeys;[72] whereas in rats depletion increased the 8-oxodG levels in the testis.[73]

Potassium bromate is a renal carcinogen and it has consistently induced 8-oxodG in the kidneys in rats.[31,74–77] This compound has been used to study preventive effects of vitamin C, glutathione, cysleine (Table II).[31] Dimethyl arsenic acid a representative arsenical and liver carcinogen induced 8-oxodG in the liver in rats.[78]

TABLE II Animals studies of prevention of oxidative DNA damage measured as 8-oxodG or by comet assay

Treatment	Species and target	Prevention	Reference
2-Nitropropane	Rat liver	Vitamin E, ellagic acid, (EGCG)	[22]
		Green tea, (EGCG)	[22]
		Benzyl selenocyanat;	[25]
± iron depletion	Rat liver	Mn and Cu depletion	[27]
	Rat liver, kidney, bone marrow, urine	Brussels sprouts	[28]
$KBrO_3$	Rat kidney	Vitamin C, glutathione, cysteine	[31]
Fe-NTA	Rat kidney	N-acetyl cysteine 2-MES,	[32]
		vitamin E	[33]
Etinyl estradiol	Rat liver	Vitamin C and E, β-carotene	[34]
Pentacholorphenol	Mouse liver	Vitamin C and E, EGCG, diallyl sulfide	[35]
NNK	Mouse lung	Green tea, EGCG	[36]
Diethylnitrosamine	Rat liver	Green tea	[37]
Aflatoxin B1	Rat liver	Selenium, desferrioxamine	[38]
Dimethylhydrazine	Rat colon	Green tea	[39]
Benzene + LPS	Mouse bone marrow	Propylene glycol*	[40]
		Dexamethasone	[41]
TPA	Mouse skin	Sarcophytol	[42]
		EGCG, tamoxifen	[43]
		Phenethyl ester, caffeic acid	[44]
Diesel particles ± fat	Mouse lung	β-carotene	[45]
Choline deficient diet	Rat liver	Vitamin C and E	[46]
		Ethionine, methionine	[47]
		Aspirin	[48,49]
		Iron depletion	[50]
		Green tea	[37]
		No effect of DPPD	[51]
Depletion of vitamin C and E	Not Guinea pig liver	No effect of high vitamin C or E	[52]
Spontaneous levels	Rat liver	No effect of vitamin E	[53]
	Rat kidney, urine, not liver or bone marrow	Brussels sprouts	[28]
	Rat liver	Food restriction	[54]

*Only comet assay; DPPD: N,N′-diphenyl-p-phenylenediamine.

REDOX CYCLING AGENTS

Redox cycling agents may generate great quantities of superoxide anions during their metabolism and would thus be expected to induce oxidative DNA damage.[61] However, menadione, one of the mostly used model compounds in this respect, failed to increase 8-oxodG levels in isolated hepatocytes despite the fact that DNA fragmentation was induced.[79] Similarly, in an *in vivo* study in rats modulation of cellular redox control by phenobarbital (for induction of cytochrome P450 reductase), dicumarol (for inhibition of quinone reductase) and phorone (for depletion of glutathione), induced 8-oxodG levels in the liver independently of the concomitant treatment with menadione.[80]

Paraquat and hydroquinone have been reported to increase the urinary excretion of 8-oxoguanine.[38] Similarly, paraquat has been reported to increase the level of 8-oxodG about 5-fold in the lung and brain and to lesser extent in the liver in rats.[81] However, in our laboratory we have seen no increase in the 8-oxodG levels in the liver and only minor and insignificant increases in the lung and brain after administration of a similar dose of paraquat which was sufficient to cause a 10% mortality in the rats

(unpublished data). The reason for this discrepancy is not clear.

Estrogens can, via catechol metabolism, undergo redox cycling generating reactive oxygen species.[82] Indeed, peroxidative metabolism of diethylstilbestrol *in vitro* caused oxidation of deoxyguanosine to 8-oxodG.[83] Several estrogens have been shown to cause oxidative DNA damage *in vivo*. In the rat ethinylestradiol treatment increased 8-oxodG levels in the liver,[34] whereas in hamsters both estradiol and diethylstilbestrol caused 8-oxodG formation in the liver and kidney.[84–86] In both species 8-oxodG formation was correlated with tumour formation and the effects could be prevented by a combination of vitamin C and B and β-carotene in the rat or vitamin C alone in hamsters.[34,86] In ovariectomised rats induction of 8-oxodG in liver DNA by 2,3,7,8-tetrachlorodibenzo-p-dioxin was reduced, suggesting that the effect is related to metabolism of endogenous estrogens by inducible cytochrome P450 enzymes, including CYP1B1.[87] Nevertheless, estrogens have profound regulatory effects on cells expressing the relevant receptors and it is thus tempting to speculate on whether the DNA damaging effects are only related to redox cycling chemistry.

Pentachlorophenol is a liver carcinogen and has been shown to cause formation of 8-oxodG in mouse liver.[35,88] Tetrachloro-p-hydroquinone, the major metabolite of pentachlorophenol, is known to autoxidise to its semiquinone radical and is likely to be responsible for this effect as shown in a separate study in mice.[89] The effects of pentachlorophenol could be prevented by oral administration of vitamin E and diallyl sulfide, whereas ellagic acid and EGCG offered partial protection and β-carotene none at all.[35]

PROCARCINOGENS REQUIRING METABOLIC ACTIVATION

A number of different standard procarcinogens or mutagens which are generally associated with DNA adduct formation after metabolic activation to reactive metabolites also induce oxidative DNA damage, at least in terms of 8-oxodG in various tissues. These compounds include classical carcinogens, such as aflatoxin, DMBA, the food mutagen benz[a]pyrene MeIQx and various nitrosamines, (Table III).[37,90–96] With the exception of one study showing no effects on hepatic 8-oxodG of three different nitrosamines,[24] all the

TABLE III Oxidative DNA damage induced by standard mutagens inflicting DNA adducts after metabolic activation

Compound	Species	Target organ(s)	Reference
Aflatoxin B_1	Rat	Liver	[90,97]
Benz[a]pyrene	Rat	Liver and kidney	[91]
DMBA	Rat	Mammary gland	[93]
	Mouse	Epidermis	[98]
Dimethylhydrazine	Rat	Colon and liver, not kidney	[99]
MeIQx	Rat	Liver	[92]
Nitrosamines			
NNK	Mouse	Lung > liver, not kidney	[94]
		Lung	[36]
		Lung and liver	[100]
		Lung and foetal liver	[101]
Diethylnitrosamine	Rat	Liver	[37]
EHEN	Rat	Kidney, not liver or lung	[95]
N-nitrosodimethylamine	Rat	Liver	[96]
		No effect in liver	[24]
N-nitrosodiethylamine	Rat	No effect in liver	[24]
N-nitrosomorpholine	Rat	No effect in liver	[24]

other studies, including 7 on nitrosamines, show induction of 8-oxodG in target tissues of these carcinogens (Table III). Some of the studies of the effects of DMBA in mouse skin and of MeIQx and N-nitrosodimethylamine in rat liver show close correlation between the formation of 8-oxodG and the development of tumours.[92,96,98] In mouse epidermis this correlation was even better than between DMBA-DNA adducts and the tumour process.[98] The consistent ability of these mutagens to induce 8-oxodG suggests that oxidative DNA damage also play a role in their carcinogenic effects. The mechanisms could involve both generation of reactive oxygen species during the metabolism of the mutagens and a consequence of cellular damage caused by their reactive metabolites. Several of the mutagens have been used to address preventive effects of selenium, desferrioxamine, vitamin E, green tea, EGCG and nonsteroidal inflammatory drugs (Table II).[37,38,94,100]

Although benzene does not generate DNA adducts it requires metabolic activation by CYP2E1 for its toxic effects. Benzene induced strand breaks and raised 8-oxodG in the target cells in the bone marrow in mice and this effect was enhanced by LPS as described below and reduced by pretreatment with propylene glycol, which inhibits metabolism by CYP2E1.[40,41,102]

PEROXISOME PROLIFERATORS

In rodents, in particular, peroxisome proliferators cause generation of substantial amounts of hydrogen peroxide.[103] Accordingly fibrates, phthalates and similarly acting compounds have consistently been shown to increase the levels of 8-oxodG in nuclear and mitochondrial DNA from the liver.[104–110] So far, however, no attempts to prevent such effects have been published.

Some haloacetates are peroxisome proliferators and some haloacetates have been shown to induce 8-oxodG formation in mouse liver.[111] However it could be demonstrated that dichloroacetate which is a peroxisome proliferator did not increase the 8-oxodG levels, whereas the brominated analogues induced 8-oxodG but not peroxisomal proliferation.

INFLAMMATION

In inflammation large quantities of reactive oxygen and nitrogen species are produced and resulting oxidative DNA damage would be expected.[3] Indeed simulation of inflammation by TPA or LPS induced a number of oxidative DNA base modifications in various cell lines, isolated human granulocytes and in co-cultured target cells,[112–115] although the formation of 8-oxodG in granulocytes by TPA has later been questioned.[116] Similarly TPA induced 8-oxodG, thymine glycol and 5-hydroxyuridine in the skin, in particular in sensitive SENCAR mice, whereas the effect was less pronounced in other mice.[42,117] The effect of TPA has been inhibited by various agents, including sarcophytol, caffeic acid phenethyl esters from propolis, EGCG and tamoxifen as shown in Table II. In transgenic mice with chronic active hepatitis 8-oxodG accumulates in the liver, presumably due to the continuous inflammatory process.[118]

The leukaemogenic effect of benzene may involve inflammatory processes and resulting oxidative DNA damage.[102,119,120] Thus, benzene can cause generation of nitric oxide and reactive oxygen species in relevant cells.[119,121,122] *In vitro* TPA enhanced benzene induced strand breaks in mouse bone marrow cells and human leukocytes whereas LPS pretreatment enhanced the benzene-induced damage in bone marrow cells assessed by the comet assay and 8-oxodG formation in mice *in vivo*.[41,123] Moreover, blocking the inflammation by dexamethasone abrogated the oxidative DNA damage.[41] Peroxynitrite and TPA activated human granulocytes can generate nitrated and hydroxylated metabolites of benzene *in vitro* and this pathway may also be relevant in the toxic mechanism.[124]

Installation of diesel exhaust particles in the trachea in mice resulted in formation of 8-oxodG in the lungs corresponding to the development of tumours.[45,125] Although diesel particles alone can generate reactive oxygen species they are also potent inducers of inflammation.[126–128] Similarly, silica particles induce inflammation and 8-oxodG in the lung of rats.[129] The 8-oxodG inducing effect of diesel particles was enhanced by a high fat diet and reduced by β-carotene.[45,125]

DIETARY INTERVENTIONS

A number of dietary manipulations can induce oxidative DNA damage. A choline deficient and amino acid defined diet has consistently induced 8-oxodG levels in rat liver.[37,46–50,130–132] Moreover, this effect may be related to carcinogenesis.[133] The choline deficient model has been used to investigate the preventive effects of a number of factors, such as iron depletion, asprin, vitamin C and E, ethione, methionine and green tea as shown in Table II.[46–50]

A diet rich in fat, particularly of unsaturated composition, may be expected to induce oxidative DNA damage, since lipid peroxidation products generated 8-oxodG in isolated DNA. However, unsaturated fatty acids had no effect on the level of 8-oxodG in cultured human lymphocytes.[134,135] In rats, high fat (24.6%) diets based on palm oil, corn oil and menhaden were compared and a significant correlation between the extent of unsaturation and 8-oxodG levels in mammary tissue was shown.[136] Moreover, the slope of the linear relationship was steeper in rats fed a diet deficient in vitamin E and selenium as compared to normal animals. However, in another rat study no difference in mammary 8-oxodG level was seen in rats fed a 20% fat diet based on lard or corn oil.[93] Similarly, no significant differences in liver 8-oxodG levels were seen between rats fed a diet based on fish oil or soybean oil with different levels of vitamin E.[137] At a fixed total energy intake, rats fed diets with either 20% or 3% corn oil had significantly lower levels of 5-hydroxyuracil in DNA from mammary gland epithelium as compared with rats fed a control diet with 5% corn oil.[138] In mice a high fat diet enhanced the induction of 8-oxodG in lung DNA by intratracheal installation of diesel particles.[45,125] In our laboratory, the urinary excretion of 8-oxodG was approximately 3-fold increased in rats fed a diet with 23% fat based on either corn oil or coconut oil as compared with rats fed normal chow with 3% corn oil (unpublished data). A high fat diet allows a large intake of energy and metabolic rate, which may lead to an increased production of reactive oxygen species.[139] Indeed, energy restriction reduces oxidative modification of both tissue DNA and proteins in rodents which may explain the reduced cancer risk and increased longevity.[54,140,141] However, such effects have not been reproduced in terms of urinary 8-oxodG excretion in humans subjected to approximately 20% energy restriction.[142] In Emory mice the urinary excretion of 8-oxodG was even increased in energy restricted animals, possibly due to a higher level of physical activity.[143]

Nutrional antioxidants may also be subject to dietary manipulations and supplementation with vitamin C and E as well as β-carotene has been used to prevent chemically-induced oxidative DNA damage with some success as shown in Table II and described above. In guinea pigs it is possible to deplete vitamin C. However, even with extensive depletion and supplementation, creating a 59-fold gradient in vitamin C concentrations in the liver no differences in 8-oxodG levels were seen in guinea pigs.[52] Similarly, depletion or extensive supplementation of vitamin E had no effect on the levels of 8-oxodG in the liver from rats or guinea pigs.[52,53]

RADIATION

Ionising radiation generates oxygen radicals and cause damage to isolated DNA and particularly

to sensitive cells in culture.[144,145] *In vivo*, however, very large doses in excess of 100 Gy are required to generate increased levels of 8-oxodG or a number of oxidised bases in DNA from mouse liver.[15,146] However, this could be due to ongoing repair as the yield per radiation dose has been reported to be much higher in terms of urinary excretion of 8-oxodG and thymidine glycol in mice.[147]

Near-ultraviolet radiation dose-dependently induced 8-oxodG levels in the epidermis of hairless mice, an effect that could be due to generation of singlet oxygen.[148] Chronic exposure to UV B radiation also increased the 8-oxodG levels but the presence of inflammation and generation of peroxynitrite suggest that as the responsible mechanism.[149]

CONCLUSION

Experimental studies provide powerful tools to investigate agents inducing and preventing oxidative damage to DNA in target organs and its role in carcinogenesis. So far, most animal experiments have concerned 8-oxodG and the new analytical techniques allowing determination of other damaged bases should be employed more extensively. Similarly, the influence of DNA repair capacity has only recently been addressed in such animal experiments.[70] Moreover, the level of oxidatively modified bases/deoxynucleosides in tissue DNA reflects a balance between the rate of damage and repair. Changes in the rate of damage may be assessed from the urinary excretion of repair products of which only a few have been studied so far.

A large number of chemical compounds, and radiation, induce oxidative DNA damage and some of these have been used successfully to demonstrate effects of various antioxidants and other preventive substances. Although extensively used, 2-NP poses several problems, particularly with respect to generation of other guanine products possibly interfering with assays and unknown effects. Similarly, the standard mutagens consistently induce 8-oxodG but also DNA adducts and they are thus not well suited for animal models of oxidative DNA damage. Redox cycling agents have not shown consistent effects, except for catechol estrogens but their hormonal effects complicate their use as model compounds in this respect. Potassium bromate, cobalt, nickel and ferric nitriloacetate may be used to induce DNA damage in the kidney, whereas other iron derivatives have very small effects. Inflammation and resulting DNA damage and their prevention appears to be an interesting field deserving experimental studies. Dietary manipulations, in particular choline deficiency and possibly a high fat content, may be used for study of oxidative damage whereas antioxidant depletion appears to have no effect. Ionising radiation requires very high doses for effects on DNA bases in animals, whereas UV can only be used on the skin. Accordingly, an ideal animal model for prevention of oxidative DNA damage has yet to be developed.

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