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REVIEW

Oxidative DNA Damage and Repair in Teratogenesis and Neurodevelopmental Deficits

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Several teratogenic agents, including ionizing radiation and xenobiotics such as phenytoin, benzo[a]pyrene, thalidomide, and methamphetamine, can initiate the formation of reactive oxygen species (ROS) that oxidatively damage cellular macromolecules including DNA. Oxidative DNA damage, and particularly the most prevalent 8-oxoguanine lesion, may adversely affect development, likely via alterations in gene transcription rather than via a mutational mechanism. Contributions from oxidative DNA damage do not exclude roles for alternative mechanisms of initiation like receptor-mediated processes or the formation of covalent xenobiotic–macromolecular adducts, damage to other macromolecular targets like proteins and lipids, and other effects of ROS like altered signal transduction. Even in the absence of teratogen exposure, endogenous developmental oxidative stress can have embryopathic consequences in the absence of key pathways for detoxifying ROS or repairing DNA damage. Critical proteins in pathways for DNA damage detection/repair signaling, like p53 and ataxia telangiectasia mutated, and DNA repair itself, like oxoguanine glycosylase 1 and Cockayne syndrome B, can often, but not always, protect the embryo from ROS-initiating teratogens. Protection may be variably dependent upon such factors as the nature of the teratogen and its concentration within the embryo, the stage of development, the species, strain, gender, target tissue and cell type, among other factors. **Birth Defects Research (Part C) 90:103–109, 2010.** © **2010 Wiley-Liss, Inc.**

Key words: oxidative DNA damage; DNA repair; reactive oxygen species; teratogens; teratogenesis; neurodevelopmental deficits

PREAMBLE

This commentary provides a brief perspective on some of the ambiguities surrounding the potential role of oxidative DNA damage and repair in structural teratogenesis and neurodevelopmental deficits and is not intended as a detailed review of this area or the broader field of developmental oxidative stress. The perspectives herein are largely illustrated by research using a limited number of model teratogens from the authors' laboratory, with apologies to the many other investigators working in this field. This is an emerging field with many questions remaining to be answered.

INTRODUCTION

General Mechanisms of Teratogenesis

For those teratogens that have been sufficiently studied, two general types of mechanisms implicated in abnormal development involve receptor-mediated and reactive intermediate-mediated processes (Fig. 1) (Wells et al., 2009a). Although studies usually focus upon one hypothesis, these general mechanisms are not mutually exclusive nor are the various different pathways that constitute these general mechanisms. For example, teratogens like the anticonvulsant drug phe-

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Abbreviations: 8-oxoG, 8-oxoguanine; ATM, ataxia telangiectasia mutated; BER, base excision repair; CSB, Cockayne syndrome B; CYPs, cytochromes P450; FPG, formamidopyrimidine glycosylase; G6PD, glucose-6-phosphate dehydrogenase; hOGG1, human oxoguanine glycosylase 1; HR, homologous recombination; NER, nucleotide excision repair; OGG1, oxoguanine glycosylase 1; PHSs, prostaglandin H synthases; ROS, reactive oxygen species; SOD, superoxide dismutase

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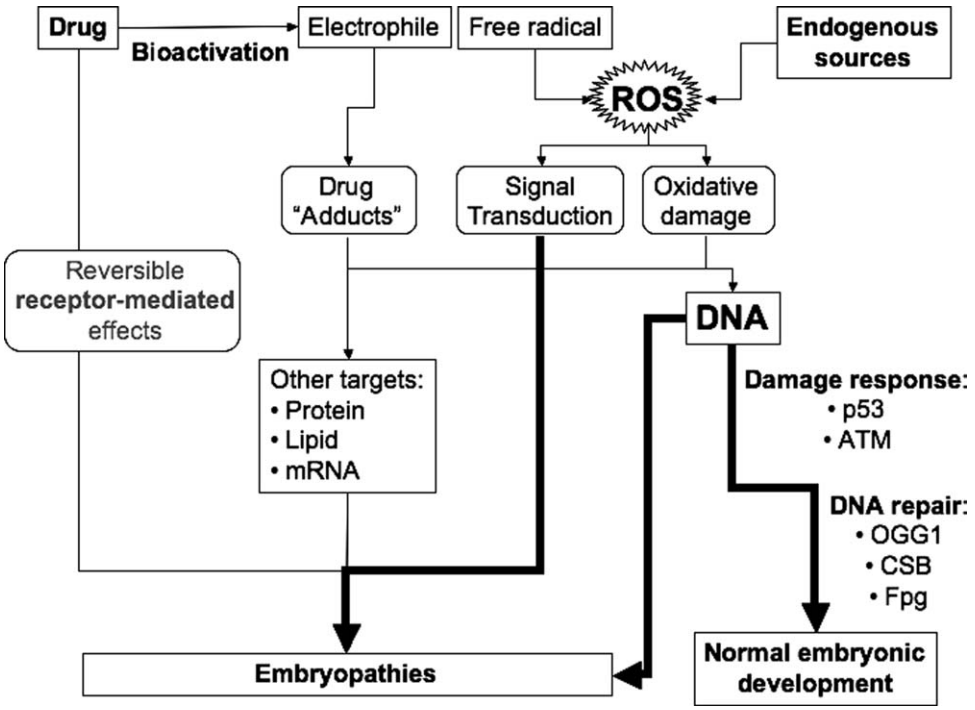

Figure 1. Competing mechanisms potentially leading to teratogenesis. Changes in teratological outcomes resulting from modifying the pathways involved in DNA damage response and repair help distinguish the role of oxidative DNA damage from oxidative damage to other cellular macromolecules (protein, RNA, lipids, and carbohydrates) as well as from ROS effects via signal transduction. Similarly, changes in teratological outcomes due to modifications in antioxidants and antioxidative enzymes help distinguish the role of ROS from mechanisms involving electrophilic xenobiotic reactive intermediates and reversible, receptor-mediated interactions (from: Wells et al., 2009b).

nytoin and the environmental chemical benzo[a]pyrene may cause birth defects in part via both receptor- and reactive intermediate-mediated mechanisms. Accordingly, results of a study implicating one mechanism often do not exclude the possibility of a lesser or greater contribution from additional mechanisms. The degree to which a given mechanism or combination of mechanisms contribute to abnormal development may vary with such factors as the nature of the teratogen, its concentration within the embryo, the stage of development, the species, strain, gender, target tissue and cell type, as well as environmental factors such as diet, stress, and concomitant exposure to xenobiotics.

Reactive Intermediates

In the case of reactive intermediates, a given teratogenic xenobiotic like phenytoin or benzo[a]pyrene may be bioactivated by enzymes like the cytochromes P450 and prostaglandin H synthases to either an unstable electrophilic or a free radical reactive intermediate, or to both (Wells et al., 2009b). The former covalently (irreversibly) binds to cellular macromolecules forming a drug–macromolecular adduct, whereas the latter can initiate the formation of reactive oxygen species (ROS), including superoxide and hydrogen peroxide, which can both alter signal transduction and form the highly reactive hydroxyl radical that can oxidatively damage cellular macromolecules (Fig. 2). The formation of drug–macromolecular adducts, oxidative macromolecular damage, and ROS-mediated altered signal transduction all may adversely affect embryonic and fetal development. Similarly, a given drug may initiate the formation of ROS via several mechanisms, including but not limited to: (1) enzymatic bioactivation to a free radical intermediate, (2) redox cycling of a quinone metabolite, (3) reperfusion effects secondary to cardiac suppression, (4) interference with the mitochondrial electron transport chain, and (5) induction or activation of enzyme systems like NADPH oxidases (NOXs, DUOXs) that produce superoxide and/or hydrogen peroxide extracellularly and/or within the cell in various subcellular locales including the nucleus (Fig. 2). As with the general mechanisms discussed above, these processes are not mutually exclusive, and the contribution of one or more may vary with the same factors. At the macromolecular level, drug teratogens may form adducts with proteins and/or DNA (or various RNAs), and drug-initiated ROS may oxidatively damage proteins, DNA/RNA, and/or lipids, the latter of which can also result in lipid products that react with proteins and/or DNA. The degree to which these various

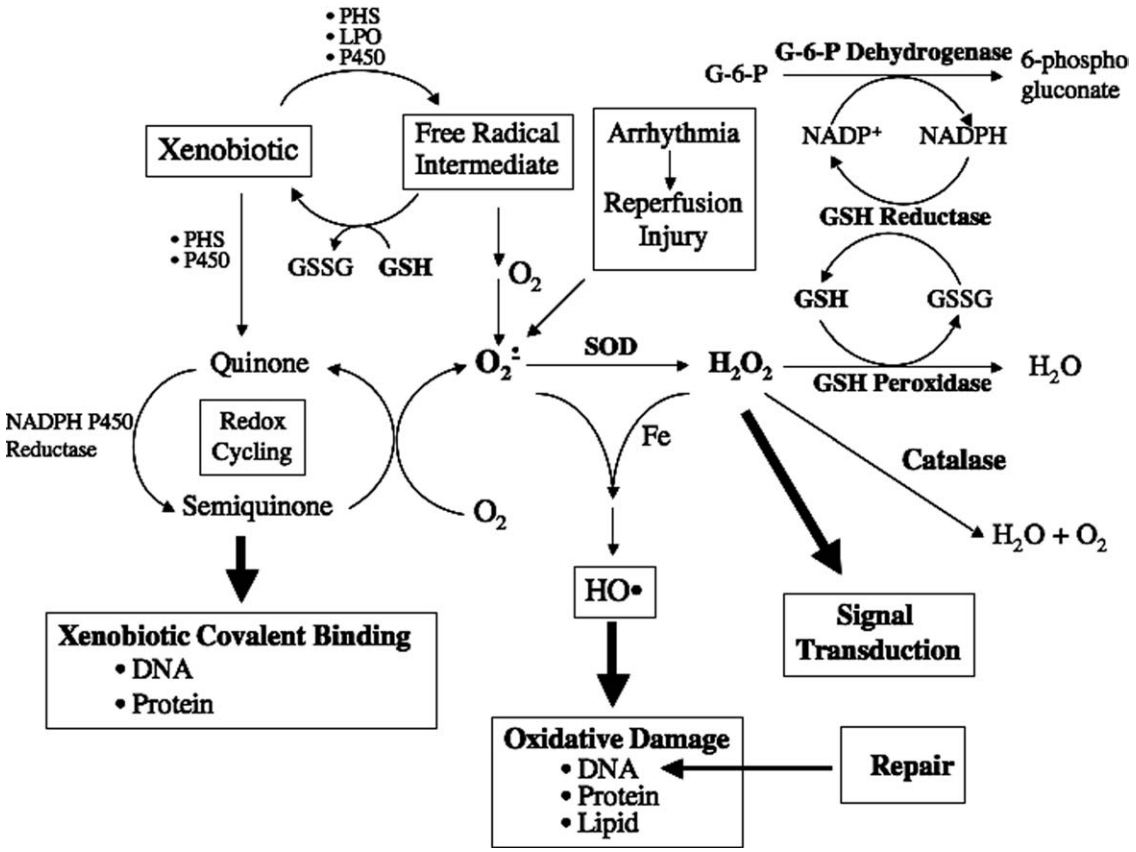

Figure 2. Biochemical pathways for the formation, detoxification, and cellular effects of xenobiotic free radical intermediates and reactive oxygen species (ROS). Abbreviations: Fe, iron; G-6-P, glucose-6-phosphate; GSH, glutathione; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide; $HO^{\bullet}$, hydroxyl radical; LPO, lipoxygenase; $NADP^{+}$, nicotinamide adenine dinucleotide phosphate; $O_2^{\bullet}$, superoxide; P450, cytochromes P450; PHS, prostaglandin H synthase, SOD, superoxide dismutase (modified from: Wells et al., 1997).

types of chemical modifications and macromolecular targets contribute to abnormal development may vary for a given teratogen as it may for the two general types of mechanisms discussed above. The same caveat holds for the multiple signal transduction cascades that may be activated by ROS.

Although such teratological mechanisms are most frequently associated with xenobiotics like drugs and environmental chemicals, they can apply to other teratogenic agents like ionizing radiation, which causes ROS formation and DNA damage including DNA double-strand breaks. Also, the contribution of ROS-initiated mechanisms elucidated in studies of chemical teratogenesis has revealed ''endogenous'' mechanisms of abnormal development that may occur in the absence of teratogen exposures, and hence constitute potential risk factors for genetic predisposition to endogenous causes of teratogenesis and *in utero* origins of adult diseases. Accordingly, the embryopathic potential of endogenous ROS in untreated animals is observed in genetically modified mice that are deficient in a key antioxidative enzyme like glucose-6-phosphate dehydrogenase (Nicol et al., 2000) or a protein sensor like ataxia telangiectasia mutated (ATM) that detects DNA damage and transduces the signal for DNA repair (Bhuller and Wells, 2006).

OXIDATIVE DNA DAMAGE

ROS and particularly hydroxyl radicals can initiate the formation of more than 20 types of oxidative DNA damage, the most prevalent of which is the 8-oxoguanine (8-oxoG) lesion that causes G:C to T:A transversion mutations capable of carcinogenic initiation (Fig. 3) and hence may be involved in the mechanism of transplacental carcinogenesis for some xenobiotics like diethylstilbestrol. However, this oxidative DNA lesion also has been causally implicated in structural and functional teratogenesis, as well as some neurodegenerative processes, for which a mutational mechanism is less likely, but may involve alterations in gene transcription (Wells et al., 2009b). Agents discussed herein that may exert their teratogenic effects in part through the formation of 8-oxoG include phenytoin, benzo[a]-pyrene, thalidomide, methamphetamine, and ionizing radiation, although the latter is associated more with DNA double-strand breaks. Although the 8-oxoG lesion has been most commonly examined, it seems likely that

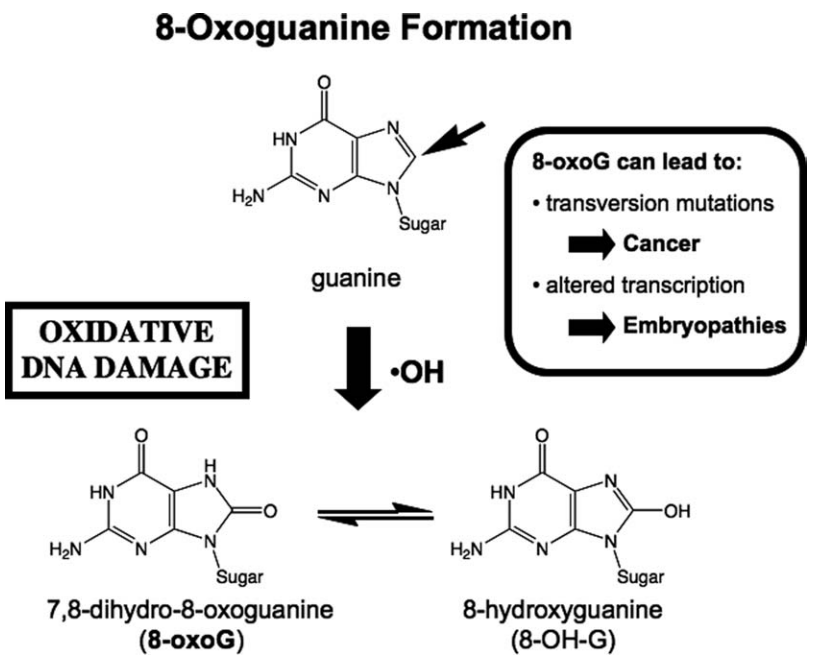

Figure 3. Reaction of hydroxyl radicals ($HO^{\bullet}$) with guanine residues of DNA to form the molecular lesion 7,8-dihydro-8-oxoguanine (8-oxoguanine, 8-oxoG). If not repaired, this oxidative damage can cause mutations and/or altered gene transcription, which may lead to cancer and/or embryopathies (from Wells et al., 2009b).

other oxidative DNA lesions, as well as lesions formed via alternative mechanisms, will prove to have embryopathic consequences, as is believed to be the case for ionizing radiation. The 8-oxoG lesion is implicated in embryopathic mechanisms in part by its elevation in embryonic tissues after agent exposure and by enhanced embryopathies in genetically modified mice that lack key proteins involved in DNA damage detection and repair transduction, like p53 and ATM, or proteins directly involved in the repair of 8-oxoG, like oxoguanine glycosylase 1 (OGG1) or Cockayne syndrome B (CSB) protein (Wells et al., 2009b). In the case of thalidomide, embryonic 8-oxoG formation is enhanced in the susceptible rabbit species, and not in the resistant mouse species, consistent with a causal role for 8-oxoG in part because both that molecular lesion and teratogenesis in rabbit embryos are blocked by pretreatment with the free radical spin trapping agent phenylbutylnitrone (Parman et al., 1999). However, other mechanisms related to oxidative stress could account for the above association, and a causal role for 8-oxoG in the mechanism of thalidomide teratogenesis remains to be established.

OXIDATIVE DNA DAMAGE DETECTION AND REPAIR

Overview

Although there is a great deal known about DNA repair, we have a limited understanding about the explicit involvement of specific repair pathways in protecting the embryo from DNA-damaging agents and particularly from ROS-initiating agents. Two of the key proteins known to be involved in detection of DNA damage and transduction of the signal for repair include p53 and ATM (Fig. 4). However, interpretations of the roles of these proteins may be confounded by the fact that, in addition to directing repair, they also transduce the signal for cellular apoptosis, and these two outcomes may have opposite consequences for embryonic development. It is possible that lower levels of embryonic DNA damage lead to the transduction of a repair signal, whereas higher levels of damage lead to apoptosis.

The major pathways involved in DNA repair include base excision repair of single-base damage, nucleotide excision repair of damage causing distortion to the DNA helix, and repair of DNA double-strand breaks by homologous recombination and nonhomologous end joining (Fig. 5). This commentary will focus on the oxidative lesions, and particularly 8-oxoG, caused by the ROS-initiating agents ionizing radiation, phenytoin, benzo[a]pyrene, and methamphetamine. The assignments to various pathways are tentative, because it is not known in many cases the full range of DNA lesions produced by a given teratogen, the intrinsic teratogenic (as distinct from mutational) efficacy of such lesions, nor even which pathway(s) in the embryo may contribute to the repair of the lesions produced. For example, there is *in vitro* evidence implicating homologous recombination as a repair pathway protecting the cell from the cytotoxicity of phenytoin, which induced both 8-oxoG formation and DNA double-strand breaks (Winn et al., 2003), and for valproic acid, which caused DNA double-strand breaks in the absence of 8-oxoG formation (Defoort et al., 2006).

DNA Damage Detection and Repair in Teratogenesis

The importance of DNA damage detection is supported by the enhanced teratogenicity of benzo[a]pyrene in p53 knockout mice (Nicol et al., 1995), and the potential embryopathic role for a ROS-mediated oxidative lesion, as distinct from a benzo[a]pyrene-DNA adduct, is suggested by the ability of this xenobiotic to increase embryonic 8-oxoG levels and the complete protection against benzo[a]pyrene embryopathies in embryo culture by the antioxidative enzyme superoxide dismutase (Winn and Wells, 1997). p53 also protects against

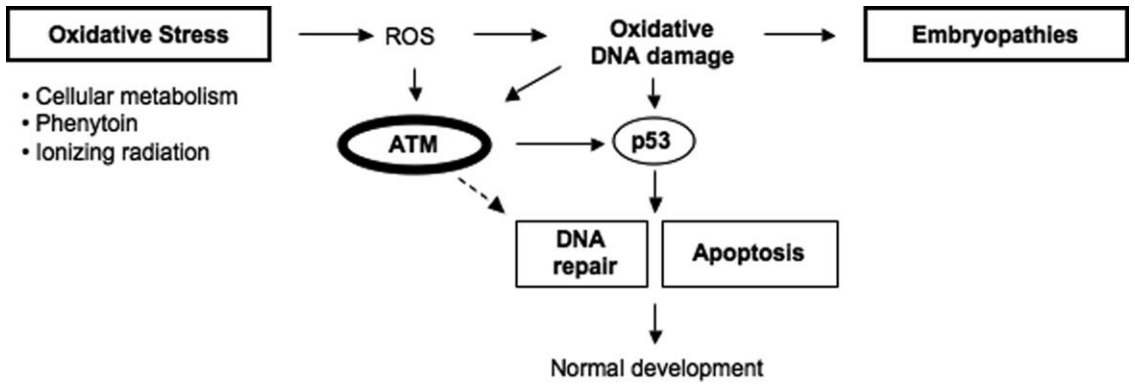

Figure 4. The potential relation of the ataxia telangiectasia mutated (ATM) and p53 proteins in the cellular DNA repair response or apoptosis after DNA damage initiated by oxidative stress (from Bhuller and Wells, 2006).

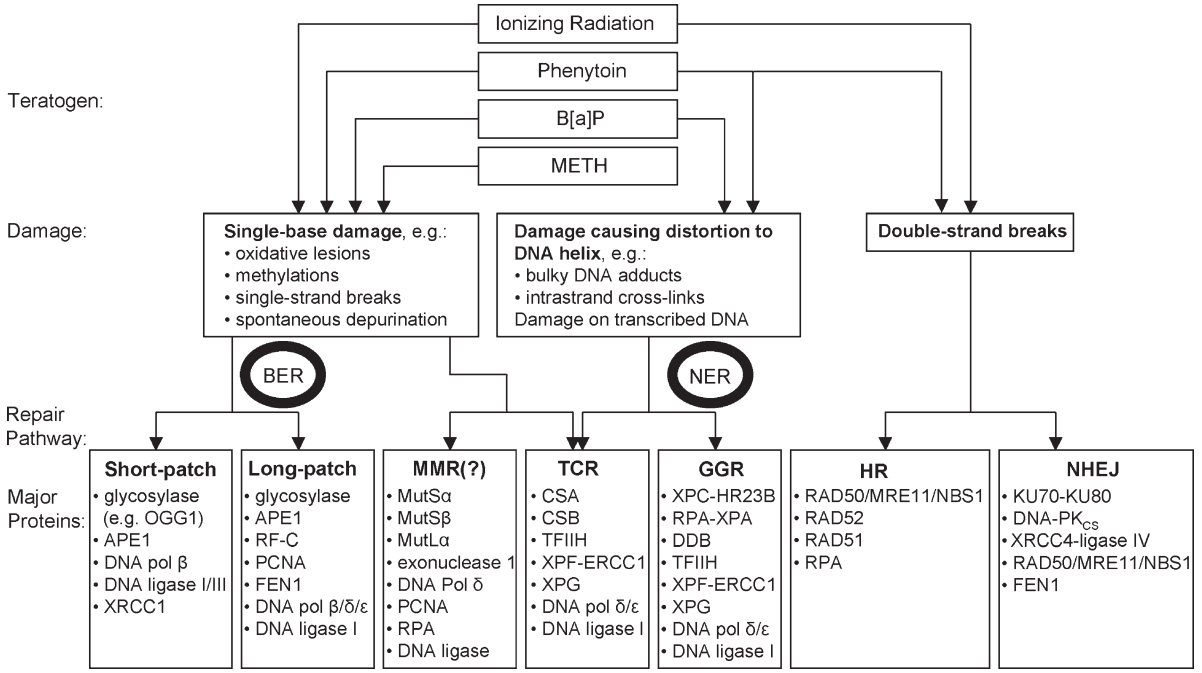

Figure 5. Major pathways for the repair of DNA damage caused by model teratogens. Abbreviations: APE1, apurinic/apyrimidinic endonuclease 1; B[a]P, benzo[a]pyrene; BER, base excision repair; CSA, Cockayne syndrome A protein; CSB, Cockayne syndrome B protein; DDB, damaged DNA-binding protein; DNA-PK_{CS}, DNA protein kinase catalytic subunit; DNA pol, DNA polymerase; ERCC1, excision repair cross-complementing 1; FEN1, flap endonuclease 1; GGR, global genome repair; HR, homologous recombination; HR23B, RAD23 homolog B; METH, methamphetamine; MMR, mismatch repair; MRE11, meiotic recombination 11; NBS1, Nijmegen breakage syndrome 1; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; OGG1, oxoguanine glycosylase 1; PCNA, proliferating cell nuclear antigen; RF-C, replication factor C; RPA, replication protein A; TCR, transcription-coupled repair; TFIIH, transcription factor IIH; XPA-G, xeroderma pigmentosum A-G; XRCC1/4, X-ray cross-complementing 1/4 (modified from Wong, 2006).

the teratogenicity of the activated analog of cyclophosphamide (Moallem and Hales, 1998), which initiates the formation of both ROS and drug–DNA adducts, so the precise mechanism of protection is unclear. Conversely, p53 knockout mice are protected against the ocular teratogenic effects of 2-chloro-2′-deoxyadenosine, presumably due to a reduced signal for apoptosis (Wubah et al., 1996), illustrating the variable role of DNA damage detection pathways in teratogenesis. A similar protective role is observed with ATM, evidenced by enhanced, gene dose-dependent teratogenicity in *Atm* knockout mice exposed to ionizing radiation *in vivo* (Laposa et al., 2004) or to phenytoin in embryo culture (Bhuller and Wells, 2006). The protective effect of ATM against phenytoin teratogenicity *in vivo* (Bhuller et al., 2006) was less comprehensive than that in embryo culture.

With respect to DNA repair, the most direct evidence of an embryopathic role for 8-oxoG comes from *Ogg1* knockout mice, which have deficient repair of the 8-oxoG lesion. In pregnant *Ogg1* knockout

mice treated with the ROS-initiating drug methamphetamine, the *Ogg1* knockout embryos exhibit enhanced levels of 8-oxoG, and the *Ogg1* knockout offspring show long-term postnatal neurodevelopmental deficits compared with their heterozygous and wild-type littermates (Wong et al., 2008). The developmental importance of OGG1 is suggested by its twofold higher activity in fetal brain and liver compared with maternal tissues (Wong et al., 2008). There can be a remarkable gender dependence in the developmental impact of ROS-initiated DNA damage and repair. For example, the enhanced susceptibility of methamphetamine-exposed *Ogg1* knockout mice was observed only in female littermates (Wong et al., 2008), whereas a gender-dependent susceptibility was not observed in repair-normal outbred CD-1 mice (Jeng et al., 2005). The mechanism for this gender dependence has yet to be determined. An embryopathic role for 8-oxoG is consistent with related studies in human embryonic kidney cells stably transfected with either human *Ogg1* (*hOgg1*) or its bacterial homolog, formamidopyrimidine glycosylase (*Fpg*) (Preston et al., 2007, 2009). Both *hOgg1*- and *Fpg*-transfected cells exhibited increased repair of the 8-oxoG lesion and resistance to the formation of 8-oxoG and cytotoxic effects caused by ROS (hydrogen peroxide) and ROS-initiating xenobiotics including menadione, platinum drugs, and phenytoin.

A protective role for DNA repair also was evident in *Csb* knockout mice, which were more susceptible than their heterozygous and wild-type littermates to fetal 8-oxoG formation and postnatal neurodevelopmental deficits caused by methamphetamine, although less so than *Ogg1* knockout mice (G.P. McCallum, A.W. Wong, and P.G. Wells, submitted). Although 8-oxoG levels were higher in the methamphetamine-exposed *Csb* knockout fetuses, CSB status did not appear to alter the *in vitro* incision of 8-oxoG catalyzed by OGG1, suggesting that CSB may contribute to 8-oxoG repair via a mechanism independent of OGG1, as has been proposed by others (Osterod et al., 2002). As with *Ogg1* mice, only female *Csb* knockout mice showed significantly enhanced susceptibility to methamphetamine-initiated neurodevelopmental deficits, although a similar nonsignificant trend was observed in male *Csb* knockout littermates. The CSB results are consistent with the OGG1 results in suggesting an embryopathic role for the 8-oxoG lesion.

EPILOGUE

There is evidence that oxidative DNA damage, and particularly the 8-oxoG lesion, may play a role in the embryopathic mechanisms of endogenous oxidative stress and ROS formation enhanced by some teratogenic agents. Although DNA repair can protect the embryo from such oxidative insults, this protection may vary with a variety of factors, including the nature of the teratogen, its concentration within the embryo, the stage of development, the species, strain, gender, target tissue and cell type, among other factors. Conversely, in some cases, as when dual pathways are transduced by proteins like p53 that regulate pathways for repair and apoptosis, activation may increase the teratogenicity of certain agents. In other cases, teratogen-enhanced formation of intermediary products in the repair pathway may prove embryotoxic. Along with a more comprehensive elucidation of the activities and relevance of specific repair pathways within the embryo, an expanded spectrum of developmental conditions, teratogens and structural and functional developmental outcomes will need to be examined to provide a proper perspective of the role of oxidative DNA damage and repair in teratogenesis.

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