Oxidative Stress-Related Mechanisms Are Associated with Xenobiotics Exerting Excess Toxicity to Fanconi Anemia Cells

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An extensive body of evidence has demonstrated the sensitivity of Fanconi anemia (FA) cells to redox-active xenobiotics, such as mitomycin C, diepoxybutane, cisplatin, and 8-methoxypsoralen plus ultraviolet irradiation, with toxicity mechanisms that are consistent with a deficiency of FA cells in coping with oxidative stress. A recent study has reported on excess sensitivity of FA complementation A group cells to chromium VI [Cr(VI)] toxicity, by postulating that a deficiency in Cr–DNA cross-link removal by FA cells and formation of Cr(VI)-associated cross-links may be the mechanism of Cr(VI)-induced cytotoxicity. However, the report failed to demonstrate any enhanced Cr uptake or, especially, any increase in Cr–DNA adducts. Thus, well-established findings on Cr(VI)-induced oxidative stress may explain excess sensitivity of FA cells to Cr(VI) in terms of its inability to cope with the Cr(VI)-induced prooxidant state. *Key words:* chromium, cisplatin, diepoxybutane, DNA adducts, Fanconi anemia, 8-hydroxy-2′-deoxyguanosine, 8-methoxypsoralen, mitomycin C, oxidative stress. *Environ Health Perspect* 111:1699–1703 (2003). doi:10.1289/ehp.6229 available via *http://dx.doi.org/* [Online 12 June 2003]

A number of organic and inorganic xenobiotics exert their toxicity via a sequence of mechanisms that relate to redox-related biotransformation (Bagchi et al. 2002; Balamurugan et al. 2002; Dusre et al. 1990; Ercal et al. 2001; Penketh et al. 2001; Quievryn et al. 2001; Sawamura et al. 1996; Seaton et al. 1995). These mechanisms include a number of distinct events that may result in direct oxidative damage, such as the formation of reactive oxygen species (ROS), the occurrence of redox couples {e.g., chromium [Cr(VI)/Cr(III)]}, and the formation of reactive intermediates of the native molecule (Dusre et al. 1990; Ercal et al. 2001; Gutteridge et al. 1984; Pagano 2002; Stohs and Bagchi 1995). As a possible alternative, the action mechanisms of some xenobiotics may interfere with some essential antioxidant systems, for example, the glutathione (GSH) system, thus resulting in a redox imbalance (Spanò et al. 1998; Vlachodimitropoulos et al. 1997).

In many instances, a combination of effects may occur, for example, related to *a*) ROS activity, *b*) modulation of redox-related activities and/or of thiol antioxidants, and/or *c*) the specific action of xenobiotic end product(s) (e.g., cross-linking with biopolymers) (Pagano 2002). The mechanistic features for any class of agents imply the extent to which these events may prevail, also depending on the individual structure analogs being considered, as well as other factors related to extra- and intracellular environment, for example, ambient oxygen levels, the endogenous redox-related activities, pH and other ionic balance, and the co-presence of other bioactive agents (hormones or other xenobiotics).

A set of xenobiotics has been reported to exert enhanced toxicity to cells from patients with Fanconi anemia (FA), a cancer-prone genetic disease (Ahmad et al. 2002; Alter 1996; Auerbach et al. 1998). The excess sensitivity of FA cells is used in FA diagnosis and is commonly attributed to the inability of FA cells to repair DNA cross-links induced by these agents. This subject has direct implications in disease definition, because the axiom of "cross-linker sensitivity" is related to the widespread opinion that FA is a DNA repair deficiency syndrome. In this commentary we review the published evidence for the involvement of several (and distinct) redox-related mechanisms in the toxicities of all the xenobiotics that have been reported to trigger enhanced sensitivity in FA cells, and we discuss the role for cross-link formation and removal.

Xenobiotics Involved in Defining FA Phenotype

Mitomycin C. Since the report by Iyer and Szybalski (1964), the requirement for redox biotransformation of mitomycin C (MMC) has been well recognized in pharmacology, because MMC must be activated by a series of redox reactions leading to semiquinone derivatives coupled with ROS formation (Dusre et al. 1990; Gutteridge et al. 1984; Penketh et al. 2001; Pritsos and Sartorelli 1986). Well-established mechanistic information has shown that MMC-associated toxicity is dependent on oxygen levels (Clarke et al. 1997; Korkina et al. 2000) and is removed by low-molecular-weight antioxidants (Raj and Heddle 1980) and by thioredoxin (Trx) overexpression (Ruppitsch et al. 1998). Recent reports demonstrate that *a*) MMC exposure results in an increase in oxidative DNA damage (Pagano et al. 2001); *b*) FA cells fail to accumulate higher MMC adduct levels in DNA than control cells (Rutherford et al. 1999; Will et al. 1998); and *c*) the ratio of MMC biadducts to monoadducts (reflective of a DNA repair defect) shows no difference in FA cells compared with normal cells (Rutherford et al. 1999). In a previous study, Clarke et al. (1997) had shown that MMC-induced apoptosis in FA complementation group C (FA-C) cells occurred under normoxic conditions (associated with redox-cycling mechanisms), whereas MMC was ineffective in causing apoptosis under hypoxic conditions (with prevailing cross-link formation).

Diepoxybutane. Diepoxybutane (DEB) toxicity may be indirectly associated with redox mechanisms because its epoxide structure implies susceptibility to GSH scavenging (Bartók and Láng 1980). Similar to MMC, DEB-associated toxicity correlates with oxygen levels (Korkina et al. 2000) and oxidative DNA damage (Pagano et al. 2001), whereas DEB-induced cytotoxicity and DNA damage are decreased by Trx overexpression (Ruppitsch et al. 1998). DEB toxicity is modulated by GSH, and in turn, DEB results in GSH depletion (Korkina et al. 2000; Spanò et al. 1998; Vlachodimitropoulos et al. 1997). Thus, a major feature of DEB-induced toxicity may be related to an imbalance in GSH levels resulting in a cellular prooxidant state.

Cisplatin. After the early report by Poll et al. (1982) showing excess sensitivity of FA cells to platinum compounds, cisplatin (CDDP)-associated toxicity has been regarded as an example of cross-linkers exerting enhanced toxicity to FA cells (Auerbach et al. 1998). Further studies, however, have provided mechanistic evidence for CDDP biotransformation leading to oxidative stress along with a cascade of redox-associated events. It has been recognized that oxidative pathways participate in the characteristic nephrotoxicity of CDDP, including

induction of heme oxygenase-1 (Schaaf et al. 2002), and CDDP-induced cytotoxicity and ROS formation are effectively counteracted by antioxidants such as α-tocopherol, vitamin C, and *N*-acetylcysteine (NAC) (De Martinis and Bianchi 2001; Schaaf et al. 2002). Treatment of myeloma cells with NAC completely blocked CDDP-dependent intracellular GSH oxidation, ROS generation, poly(ADP-ribose) polymerase cleavage, and apoptosis (Godbout et al. 2002). Bauman et al. (1991) reported a protective role of metallothionein, a scavenger of hydroxyl radicals, against a number of oxidative stress–associated xenobiotics, including CDDP.

Waisfisz et al. (2002) have demonstrated that *p21^wafl/Cip1*, an oxidative stress regulatory gene (Esposito et al. 1998), is overexpressed in lymphoblastoid FA cells and is very sensitive to CDDP. *p21^wafl/Cip1* is known to be a key regulator of G1 cell-cycle arrest and has been recently linked to G2 cell-cycle regulation (Taylor and Stark 2001). This observation fits well with the previously observed up-regulation of apoptotic regulatory p53 protein and cyclin B proteins, inactivation of CDC2 kinase, and cell-cycle arrest, which follows exposure to low doses of cross-linking agents in FA cells (Kruyt et al. 1996, 1997; Kupfer and D'Andrea 1996). Also p53, cyclin B, and CDC2 kinase are known to be involved in oxidative stress pathways (Barnouin et al. 2002; Chen et al. 2002; Chuang et al. 2002).

Hence, the literature provides cellular and molecular evidence for a direct involvement of redox pathways in CDDP-associated toxicity mechanisms.

8-Methoxypsoralen and ultraviolet A. Early studies pointed to the excess sensitivity of FA cells to 8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA) irradiation (PUVA), and the results were construed as due to inability to cope with 8-MOP cross-link formation (Moustacchi and Diatloff-Zito 1985). The combination of PUVA, however, is associated with production of ROS that react subsequently with DNA, lipids, and proteins and cause a decrease in cellular antioxidative capacity (Reinheckel et al. 1999). Both UVA irradiation and PUVA treatment induced significant changes in the distribution of arachidonic acid and increased the liberation of arachidonic acid from membrane phospholipids, with a significant increase in the formation of thiobarbituric acid–reactive material. The activities of the antioxidant enzymes catalase and superoxide dismutase were also significantly decreased by UVA (Punnonen et al. 1991). PUVA-associated toxicity has been shown to cause GSH depletion (d'Ischia et al. 1989), which in turn involves an oxidative stress condition.

Chromium(VI). In a recent article, Vilcheck et al. (2002) reported on excess sensitivity of FA complementation A group (FA-A) cells to Cr(VI)-induced toxicity and apoptosis.

Cell growth and apoptosis were measured by phosphatidylserine translocation and caspase-3 activation. Both the induction of apoptosis and of clonogenicity showed significantly increased sensitivity to Cr(VI) in FA-A cells versus control fibroblasts. However, no increase in Cr(VI) uptake or in Cr–DNA adducts was detected in FA-A cells versus control cells.

Vilcheck et al. (2002) relied on the assumptions that FA phenotype and Cr-associated toxicity should be based on *a)* excess sensitivity to DNA cross-linking agents, suggesting "a general defect in the repair of DNA cross-links" and *b)* the ultimate events in Cr-associated toxicity, namely, the formation of DNA lesions, such as DNA–Cr–DNA interstrand cross-links (Bridgewater et al. 1998; Mattagajasingh and Misra 1999; O'Brien et al. 2001; Singh et al. 1998). However, this study provides support to the body of evidence relating oxidative stress both to FA phenotype and to Cr toxicity.

An extensive body of literature has provided evidence for the role of multiple redox-related events in Cr-associated toxicity, as summarized in Table 1. These events include both direct and indirect mechanisms related

to oxidative stress, for example, Cr-induced depletion of GSH and other thiol-based antioxidants (Nguyen-nhu and Knoops 2002; O'Brien et al. 2001; Quievryn et al. 2001); protective effects of catalase, melatonin, or NAC (Cabrer et al. 2001; Izzotti et al. 1998; Shi et al. 1999); ROS formation (Bagchi et al. 2001; Hassoun and Stohs 1995; Izzotti et al. 1998; Tsou et al. 1996); nitric oxide production (Hassoun and Stohs 1995); and lipid peroxidation (Bagchi et al. 1995, 1997). The eventual formation of Cr adducts to DNA, or to proteins and DNA, may be viewed as an ultimate step in a set of redox-dependent mechanisms (Blasiak and Kowalik 2000; Hodges et al. 2001).

As shown in Tables 2 and 3, the xenobiotics commonly used in FA diagnosis (MMC, DEB), or otherwise associated with excess sensitivity in FA cells (CDDP, PUVA), display distinct mechanisms of toxicity that, however, involve redox-dependent actions interrelated with ROS formation and/or oxygen levels, and/or modulation of antioxidant levels, and/or antioxidant enzyme activities.

These mechanistic features, conversely, may suggest a redox imbalance in FA cells, as far as

Table 1. Summary of the published evidence for the association of Cr toxicity with oxidative stress.

End points	Observations	References
Oxidative stress and tissue damage	↑ DNA damage, apoptosis, altered gene expression; urinary lipid metabolites; nitric oxide production and ROS formation	Bagchi et al. (1995; 2002), Hassoun and Stohs (1995), Travacio et al. (2000)
p53 expression	↑ Oxidative damage in p53-null mice	Bagchi et al. (2001)
ROS- and Src-dependent apoptosis	Induced by Cr(III) complexes involved in ROS generation	Balamurugan et al. (2002)
Lipid and DNA oxidation	Counteracted by melatonin	Cabrer et al. (2001)
8-OHdG *in vivo* formation	Suppressed by NAC	Tsou et al. (1996), Izzotti et al. (1998), Hodges et al. (2001)
GSH- and cysteine-associated protection toward DNA cross-linking	Preferential cross-linking of GSH to DNA; *in vivo* role of cysteine in reduction of Cr(VI)	O'Brien et al. (2001), Quievryn et al. (2001)

Table 2. Summary of the published evidence for the redox-dependent mechanisms in MMC- and DEB-associated toxicity.

End points	Observations	References
MMC		
FA-group C cell apoptosis	Modulated by oxygen levels	Clarke et al. (1997)
Biotransformation to toxic derivatives	Effected by a set of redox activities; associated with ROS (·OH) formation	Iyer and Szybalski (1964), Gutteridge et al. (1984), Pritsos and Sartorelli (1986), Dusre et al. (1990), Penketh et al. (2001)
Embryotoxicity	Modulated by oxygen levels; ↑ 8-OHdG formation	Korkina et al. (2000), Pagano et al. (2001)
Chromosomal instability	Modulated by low-molecular-weight antioxidants, thioredoxin over-expression, and redox-related enzymes	Nordenson (1977), Raj and Heddle (1980), Ruppitsch et al. (1998)
DEB		
Cytotoxicity and embryotoxicity	Modulated by GSH levels, oxygen levels, and thioredoxin overexpression; ↑ 8-OHdG formation	Vlachodimitropoulos et al. (1997), Spanò et al. (1998), Ruppitsch et al. (1998), Korkina et al. (2000), Pagano et al. (2001)

8-OHdG, 8-hydroxy-2′-deoxyguanosine.

they display a prooxidant state making them unable to cope with prooxidant xenobiotics.

Prooxidant State in FA Phenotype

FA is a genetic disorder associated with bone marrow failure, chromosomal instability, and poor prognosis (Alter 1996; Auerbach et al. 1998). FA shows a progression to aplastic anemia, excess risk of malignancy (mainly nonlymphocytic leukemia), a set of typical malformations, and altered skin pigmentation. A total of eight complementation groups have been identified, and six genes (*FANCA, FANCC, FANCD2, FANCE, FANGF, FANCG*) have been cloned so far (Tamary et al. 2002). Diagnosis is performed by excess FA cell sensitivity to either DEB or MMC that induces high rates of chromosomal breaks (Auerbach and Wolman 1976). A line of molecular evidence has related at least two FA gene–encoded proteins with redox pathways. The protein encoded by the Fanconi gene *C* (*FANCC*) has been shown to associate with redox-related activities, namely, NADPH cytochrome P450 reductase (Kruyt et al. 1998) and glutathione *S*-transferase (Cumming et al. 2001). A recent study has provided evidence that the FANCG protein interacts with cytochrome P450 2E1 protein, an activity also known to be involved in redox biotransformation of xenobiotics (Futaki et al. 2002). Previous indirect evidence for an abnormal regulation of xenobiotic biotransformation in FA cells was provided by Joenje and Oostra (1986), who reported excess sensitivity of FA cells to cyclophosphamide without S-9 metabolic activation (Madle 1981). This finding suggested that FA cells, unlike non-FA cells, were endowed with a complete set of phase 1 activities for cyclophosphamide biotransformation.

The relationship of the FA phenotype with a prooxidant state was first suggested by Nordenson (1977). A consistent body of evidence has been accumulated since then, both related to phenotypic redox abnormalities and related to the involvement of FA proteins in redox pathways (Ahmad et al. 2002; Bogliolo et al. 2002; Pagano et al. 1998; Pagano and Korkina 2000; Pagano and Youssoufian 2003), as summarized in Table 4. FA cells are characterized by *a*) excess oxidative DNA damage [8-hydroxy-2′-deoxyguanosine (8-OHdG)] in hydrogen peroxide-exposed FA cells (Takeuchi and Morimoto 1993), and in freshly drawn white blood cells from FA patients (Degan et al. 1995); *b*) increased oxygen sensitivity (Joenje et al. 1981) and sensitivity to free iron (Poot et al. 1996); *c*) correction of chromosomal abnormalities by either antioxidant enzymes or by low-molecular-weight antioxidants (Dallapiccola et al. 1985; Nordenson 1977; Raj and Heddle 1980); *d*) excess plasma levels of clastogenic factor (Emerit et al. 1995) and of tumor necrosis factor-α (TNF-α; Dufour et al. 2003; Schulz and Shahidi 1993); *e*) involvement of redox-active cytokines, such as TNF-α and interferon-γ (Dufour et al. 2003; Pearl-Yafe et al. 2003), and of inducible nitric oxide synthase (Hadjur and Jirik 2003); and *d*) an involvement of Trx, which corrects MMC and DEB sensitivity in FA cells (Ruppitsch et al. 1998) and occurs in lower-than-normal levels in FA fibroblasts (Kontou et al. 2002). Together, a well-established body of evidence on FA cellular phenotype, along with the roles for some FA proteins in redox pathways (Bogliolo et al. 2002; Pagano and Youssoufian 2003), combine to support a central role for oxidative stress both in FA cellular phenotype and in FA protein functions.

Conclusions and Suggestions

Well-established literature points to the involvement of redox-dependent mechanisms in the toxicity of MMC, DEB, CDDP, PUVA, and Cr(VI). The formation of DNA or protein–DNA cross-links may be viewed as the outcome of redox reactions coupled with ROS formation, antioxidant (e.g., GSH) depletion, and a multifaceted modulation of redox-related activities.

When these xenobiotics interact with FA cells, which are in turn characterized by a multiple prooxidant state, one should expect a more pronounced toxicity compared with control cells, due to FA cell deficiency in withstanding oxidative stress. At the same time, the failure to detect excess adduct formation after Cr(VI) (Vilcheck et al. 2002) or MMC (Rutherford et al. 1999) exposure does not support a deficiency in removing damaged DNA bases (i.e., in DNA repair).

In the same prospect, the use of FA cells may be envisioned in forthcoming studies designed to investigate the action mechanisms of redox-related environmental agents or pharmaceutical drugs.

Table 3. Summary of the published evidence for the redox-dependent mechanisms in CDDP- and PUVA-associated toxicity.

End points	Observations	References
CDDP		
Nephrotoxicity	Induction of heme oxygenase-1	Schaaf et al. (2002)
ROS formation	Inhibited by antioxidants, α-tocopherol, vitamin C, and NAC	Schaaf et al. (2002), De Martinis and Bianchi (2001)
GSH oxidation, ROS formation, and apoptosis	Suppressed by NAC, enhanced by methylglyoxal	Godbout et al. (2002)
Overexpression of oxidative stress-related genes	↑ Metallothionein (*in vivo*); ↑ *p21*$^{waf1/Cip1}$	Bauman et al. (1991), Waisfisz et al. (2002)
PUVA		
ROS formation	Reactions with DNA, lipids, and proteins; ↓ Cellular antioxidant capacity; ↑ TBARS	Reinheckel et al. (1999), Punnonen et al. (1991)
Antioxidant enzyme expression	↓ Catalase and superoxide dismutase	Punnonen et al. (1991)
GSH oxidation	O_2-dependent effect	d'Ischia et al. (1989)

TBARS, thiobarbituric acid-reactive substances.

Table 4. Summary of the published evidence reporting on redox abnormalities in FA cellular phenotype.

Observed outcomes	Tested/analyzed materials	References
↓ Chromosomal instability by Cu/ZnSOD, catalase, low-molecular-weight antioxidants, hypoxia (5% O_2)	Lymphocytes	Nordenson (1977), Raj and Heddle (1980), Dallapiccola et al. (1985), Joenje et al. (1981)
5% O_2 corrects cell cycle arrest; free iron sensitivity	Lymphoblastoid cell lines	Poot et al. (1996)
↑ 8-OHdG formation; sensitivity to H_2O_2; ↓ catalase expression	Lymphoblastoid cell lines	Takeuchi and Morimoto (1993)
↑ Levels of 8-OHdG; ↑ ROS formation; correlated with chromosomal instability	Freshly drawn WBCs from patients, siblings, and their parents	Degan et al. (1995)
↑ Clastogenic factor	Plasma from patients and parents	Emerit et al. (1995)
↑ TNF-α and IFN-γ related to iNOS in causing myelosuppression	Plasma and WBCs from patients	Schulz and Shahidi (1993), Pearl-Yafe et al. (2003), Dufour et al. (2003), Hadjur and Jirik (2003)
Trx overexpression corrects MMC and DEB sensitivity; lower-than-normal Trx levels	Fibroblast cultures	Ruppitsch et al. (1998) Kontou et al. (2002)

Abbreviations: IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthase; Cu/ZnSOD, copper/zinc superoxide dismutase, catalase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; TNF-α, tumor necrosis factor-α; WBCs, white blood cells.

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