Carcinogenicity in humans

Exposure to 4-aminobiphenyl, o-toluidine, 2-naphthylamine, and benzidine (Fig. 2.1) has been consistently associated with the induction of cancer of the urinary bladder in humans. This association is based upon occupational exposures, primarily of workers in the rubber and dye industries (IARC, 2010, 2012a). Similarly, occupational exposure to 4,4′-methylenebis(2-chloroaniline) (MOCA; Fig. 2.1), a curing agent for polyurethane pre-polymers, causes cancer of the bladder in humans, although the epidemiological data are not as strong as those for the other agents (IARC, 2010, 2012a).

Cigarette smoke contains 4-aminobiphenyl, o-toluidine, and 2-naphthylamine, and tobacco smoking causes cancer of the bladder in humans (IARC, 1986, 2004, 2010, 2012a, b). The contribution of 4-aminobiphenyl, o-toluidine, and 2-naphthylamine to the induction of smoking-related cancer of the bladder is confounded by the presence of numerous other carcinogens, including carcinogenic aromatic amines, in tobacco smoke. Cigarette smoking also causes other cancers (e.g. cancer of the lung, oral cavity, and pancreas, and possibly breast cancer), but at present it is unclear whether these cancers can be attributed to 4-aminobiphenyl, o-toluidine, 2-naphthylamine, or other aromatic amines. Hair dyes are an additional source of exposure to 4-aminobiphenyl and o-toluidine (IARC, 2010, 2012a; Lizier and Boldrin Zanoni, 2012).

Exposure to phenacetin (Fig. 2.1), through its use as an analgesic, causes cancer of the kidney and ureter in humans (IARC, 2012c). Chlorornaphazine (Fig. 2.1), a chemotherapeutic agent that has been used for the treatment of Hodgkin lymphoma and for the control of polycythæmia vera, causes cancer of the bladder in humans, presumably due to metabolism to 2-naphthylamine (IARC, 2012c). An additional source of human exposure to o-toluidine is from the anaesthetic prilocaine (Fig. 2.1) (IARC, 2010, 2012a).

Exposure to herbal remedies prepared from plant species of the genus Aristolochia has been causally...
associated with the induction of urothelial cancer in humans (IARC, 2002, 2012c). The induction of urothelial tumours has been attributed to the presence of aristolochic acid I and aristolochic acid II (Fig. 2.1).

**Metabolism and DNA adduct formation**

4-Aminobiphenyl, o-toluidine, 2-naphthylamine, and MOCA are metabolized to electrophilic N-hydroxyarylamines by hepatic cytochrome P450 enzymes (IARC, 2010, 2012a, c). The N-hydroxyarylamines undergo acid-catalysed reactions with DNA to form a variety of DNA adducts. With the exception of MOCA, C8-substituted deoxyguanosine adducts are typically the major products, along with smaller amounts of N2- and N6-substituted deoxyguanosine and N6-substituted deoxyadenosine adducts (Fig. 2.3); with MOCA, only C8-substituted deoxyadenosine adducts have been detected (IARC, 2010, 2012a). These DNA adducts can also be formed from reactive esters of N-hydroxyarylamines (e.g. N-sulfoxoyarylaminles and N-acetoxyarylamines). Benzidine, which has two amino groups, also forms a C8-substituted deoxyguanosine adduct via a pathway involving an initial N-acetylation followed by N-hydroxylation of the remaining amino function (Fig. 2.3).

The carcinogenic activity of aromatic amines in the bladder in humans has been attributed to an initial N-hydroxylation, catalysed by hepatic cytochrome P450 enzymes, followed by transport of the N-hydroxyarylamines to the bladder as either aglycones or N-glucuronide conjugates (Bois et al., 1995). In the bladder lumen, the N-hydroxyarylamine N-glucuronides can undergo acid-catalysed hydrolysis to release the N-hydroxyarylamines, which can enter the bladder epithelium and react with DNA either directly or after esterification. DNA adducts derived from 4-aminobiphenyl, o-toluidine, benzidine, and MOCA have been detected in bladder tissue or exfoliated bladder cells from exposed individuals (IARC, 2010, 2012a; Böhm et al., 2011; Lee et al., 2014). With the exception of MOCA, which forms only C8-substituted deoxyadenosine adducts, the major – if not the only – adduct detected in each instance was a C8-substituted deoxyguanosine adduct. The importance of urinary acidity for the hydrolysis of the N-hydroxyarylamine N-glucuronides and perhaps for the reaction of the N-hydroxyarylamines with urothelial DNA has been demonstrated by the positive correlation between urinary acidity and the levels of benzidine DNA adducts in exfoliated bladder cells from exposed workers (Rothman et al., 1997). Additional support for this mechanism comes from the observation of a positive
correlation between urinary acidity and the incidence of bladder cancer in smokers (Alguacil et al., 2011).

The major metabolic activation pathway for aristolochic acid I and aristolochic acid II involves nitro reduction, followed by cyclization to give N-hydroxyaristolactams, which – in contrast to other N-hydroxyarylamides – do not appear to require additional activation to react with DNA (Stiborová et al., 2011, 2013). Nonetheless, N-hydroxyaristolactams have been shown to serve as substrates for human sulfotransferases, particularly sulfotransferase family cytosolic 1B member 1 (SULT1B1), forming highly reactive N-sulfoxo derivatives (Sidorenko et al., 2014). The major adducts resulting from the N-hydroxyaristolactams are N2-substituted deoxyguanosines and N6-substituted deoxyadenosines (Fig. 2.4) (IARC, 2002, 2012c).

DNA adducts derived from aristolochic acids have been detected in renal tissue from patients who had been exposed to aristolochic acid-containing herbal products and from individuals who had consumed wheat grains contaminated with Aristolochia (IARC, 2002, 2012c; Chen et al., 2012; Jelaković et al., 2012; Schmeiser et al., 2012, 2014; Yun et al., 2013, 2014). Typically, the major lesion detected is an N6-deoxyadenosine adduct derived from aristolochic acid I, accompanied by smaller amounts of a similar adduct derived from aristolochic acid II and an N2-deoxyguanosine adduct derived from aristolochic acid I.

**Alterations in the TP53 tumour suppressor gene in humans**

Mutations in the TP53 tumour suppressor gene have been found in approximately 50% of all bladder cancers in humans (Petitjean et al., 2007), with G:C base substitution mutations occurring to a greater extent than A:T base substitution mutations.

TP53 gene mutations have been detected in bladder cancer patients exposed occupationally to 4-aminobiphenyl, 2-naphthylamine, and/or benzidine (Sørlie et al., 1998). The mutations occurred exclusively in higher-grade tumours (grades 2 or 3, i.e. moderately or poorly differentiated) and only at G:C base pairs.

Mutant p53 protein has also been detected in workers exposed occupationally to benzidine (Xiang et al., 2007). The occurrence and the amount of mutant protein were positively correlated with the level of benzidine exposure and the extent of neoplastic changes in exfoliated urothelial cells.

Urothelial tumours arising from exposure to aristolochic acids have been consistently shown to carry mutations in the TP53 tumour suppressor gene, of which the most common mutation is an A → T transversion.
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mutation (reviewed in IARC, 2002, 2012c; Hollstein et al., 2013; see also Schmeiser et al., 2012; Chen et al., 2013; Hoang et al., 2013; Poon et al., 2013; Aydin et al., 2014). These mutations have been demonstrated in tumour tissue from patients who had consumed herbal preparations containing aristolochic acids and in urothelial cancer tissues of patients from regions with a high incidence of endemic (Balkan) nephropathy due to consumption of grains contaminated with *Aristolochia*. The presence of A \(\rightarrow\) T transversion mutations is consistent with the observation that the major lesion detected in patients is an \(N^6\)-deoxyadenosine adduct derived from aristolochic acid I.

**Tumour sites and the mechanism of tumour induction in experimental animals**

4-Aminobiphenyl, 2-naphthylamine, and MOCA are bladder carcinogens in dogs (IARC, 2010, 2012a). Bladder tumours also occur in mice treated with 4-aminobiphenyl. As with humans, the induction of bladder tumours in dogs is thought to result from hepatic \(N\)-hydroxylation, transport of the \(N\)-hydroxyarylamines to the bladder as either aglycones or \(N\)-hydroxyarylamine \(N\)-glucuronides, and subsequent hydrolysis of the \(N\)-hydroxyarylamine \(N\)-glucuronides in the bladder lumen to release the \(N\)-hydroxyarylamines.

Support for this mechanism comes from the observation that the DNA lesions detected in the bladders of dogs treated with 4-aminobiphenyl or MOCA appear to be C8-substituted deoxyguanosine and deoxyadenosine adducts that are identical to the DNA adducts detected in bladder tissues or exfoliated bladder cells from humans exposed to these carcinogens (IARC, 2010, 2012a). 2-Naphthylamine DNA adducts detected in the bladders of dogs exposed to 2-naphthylamine are entirely consistent with a mechanism involving the formation of \(N\)-hydroxy-2-naphthylamine (IARC, 2010).

In contrast to what is observed in humans, benzidine in not a bladder carcinogen in dogs. This lack of carcinogenicity has been attributed to the inability of dogs to \(N\)-acetylate aromatic amines (IARC, 2010). With most aromatic amines, \(N\)-acetylation appears to be required to give \(N\)-acetylbenzidine, which undergoes a subsequent \(N\)-hydroxylation of the second amino function. This metabolic pathway occurs in humans but not in dogs.

4-Aminobiphenyl, \(o\)-toluidine, 2-naphthylamine, benzidine, benzidine-based dyes, and MOCA induce hepatocellular tumours in mice (IARC, 2010, 2012a). DNA adducts derived from 4-aminobiphenyl and benzidine have been examined in liver tissue from exposed mice, and the major DNA lesions detected in each instance were C8-substituted deoxyguanosine adducts, consistent with formation of an \(N\)-hydroxyarylamine intermediate (IARC, 2010). In rats, DNA adducts derived from benzidine-based dyes and MOCA have been examined in the liver, which is also a target tissue for these...
carcinogens, and again the major DNA adducts detected in each instance were consistent with formation of an N-hydroxyaryamine intermediate (IARC, 2010).

In mice, there appears to be a balance between hepatic N-acetylation, which is considered to be a detoxification step, and hepatic N-hydroxylation, which is considered to be an activation step. Should N-hydroxylation occur, the N-hydroxyarylamines can be further activated by hepatic O-acetylation to yield O-acetoxyarylamines, which can give rise to the DNA adducts detected in liver tissue (IARC, 2010).

C8-substituted deoxyguanosine adducts have also been detected in the bladder DNA of mice treated with 4-aminobiphenyl (Poirier et al., 1995). These adducts presumably arise from hepatic N-hydroxylation and possibly O-acetylation of N-hydroxy-4-aminobiphenyl in the bladder epithelium.

The carcinogenicity of aristolochic acids has been assessed in rats and to a lesser extent in mice and rabbits, primarily by oral dosing (IARC, 2002, 2012c). Aristolochic acid I and mixtures of aristolochic acids I and II consistently induce tumours of the forestomach in rats. Tumours of the kidney have been reported to occur sporadically. Mice treated with mixtures of aristolochic acids I and II develop tumours of the forestomach, kidney, and lung. In rabbits, mixtures of aristolochic acids I and II administered intraperitoneally are associated with tumours of the kidney, ureter, and peritoneal cavity.

DNA adducts derived from aristolochic acid I and aristolochic acid II have been detected in target tissues in mice (forestomach, kidney, and lung), rats (forestomach and kidney), and rabbits (kidney) (IARC, 2002, 2012c; Debelle et al., 2003; Gillerot et al., 2003; Dong et al., 2006; Mei et al., 2006; Shibutani et al., 2007; Chan et al., 2008; Rosenquist et al., 2010; Shibutani et al., 2010; Baudoux et al., 2012; McDaniel et al., 2012; Wang et al., 2012a; Yun et al., 2013, 2014). Typically, three DNA adducts are detected: an N6-deoxyadenosine adduct derived from aristolochic acid I, an N6-deoxyadenosine adduct derived from aristolochic acid II, and an N7-deoxyguanosine adduct derived from aristolochic acid I.

Oncogene alterations in experimental animals

Transversion mutations at codon 61 of the H-Ras oncogene (CAA → AAA) have been observed in the livers of mice treated with 4-aminobiphenyl (IARC, 2010, 2012a). G → T transversion mutations in the cII transgene have been detected in the livers and bladders of transgenic mice treated with 4-aminobiphenyl (Wang et al., 2012b; Yoon et al., 2012). The occurrence of these mutations at G:C base pairs is consistent with the observation that the major DNA adduct detected in target tissues...
after exposure to 4-aminobiphenyl is a C8-substituted deoxyguanosine adduct.

Transversion mutations at codon 61 of the H-Ras oncogene (CAA → CTA) have been detected in tumours from rats and mice fed mixtures of aristolochic acids I and II and/or aristolochic acid I (IARC, 2002, 2012c; Wang et al., 2011, 2012a). A → T transversion mutations have also been detected in the cII transgene of rats and the cII and lacZ transgenes of mice treated with mixtures of aristolochic acids I and II, and in the gpt transgene of mice treated with aristolochic acid I or aristolochic acid II (IARC, 2012c; McDaniel et al., 2012; Xing et al., 2012). The occurrence of these mutations at A:T base pairs is consistent with the observation that the major DNA lesions detected in target tissues after exposure to aristolochic acids are N6-substituted deoxyadenosine adducts.

Summary

In humans, exposure to aromatic amines and aristolochic acids that are IARC Group 1 carcinogens has been associated with induction of tumours of the urinary tract. With aromatic amines, the primary tumour site is the bladder; with aristolochic acids, the primary site for tumour formation is the kidney. Experimental animals treated with aromatic amines or aristolochic acids develop tumours of the urinary tract; tumours also arise in other tissues, primarily the liver.

Aromatic amines and aristolochic acids that are IARC Group 1 carcinogens are metabolized by amine oxidation (in the case of aromatic amines) or nitro reduction (in the case of aristolochic acids) to N-hydroxyarylamine metabolites in both humans and experimental animals. These N-hydroxyarylamine intermediates can react directly with DNA or be further activated by O-esterification to give rise to DNA adducts, predominantly at deoxyguanosine (primarily with aromatic amines) and deoxyadenosine (primarily with aristolochic acids), in tumour target tissues of humans and experimental animals.

Mutations of the TP53 tumour suppressor gene consistent with the major DNA adducts derived from aromatic amines and aristolochic acids have been detected in tumours from exposed humans. Similarly, mutations of the H-Ras oncogene consistent with the major DNA adducts derived from aromatic amines and aristolochic acids have been found in target tissues of experimental animals.

Disclaimer

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References


