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2,4-Diaminotoluene (2,4-DAT)-induced DNA damage, DNA repair and micronucleus formation in the human hepatoma cell line HepG2

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Abstract

2,4-Diaminotoluene (2,4-DAT) is a widely used industrial intermediate and human exposure is possible in the dye and plastics industries. We investigated the genotoxicity of the environmental pollutant, 2,4-DAT, in human HepG2 cells using the unscheduled DNA synthesis (UDS) test, the micronucleus (MN) assay and single-cell gel electrophoresis (SCGE). 2,4-DAT was first tested by the RNA synthesis inhibition test as a cytotoxicity assay: the IC_{50} of 2,4-DAT was 5.2 mM after 20 h of exposure. The compound had a genotoxic effect at concentrations from 1.45 to 6.80 mM in both micronucleus and comet assays. In the micronucleus assay, the number of MN/1000 BNC was 3.5 times higher at a concentration of 6.80 mM 2,4-DAT than in the negative control. At the same concentration, DNA migration (SCGE) showed an Olive tail moment (OTM) of 3.56 ± 0.45, as compared to 0.19 ± 0.02 for the negative control. The UDS test detected genotoxic effects at lower concentrations than did the other assays (0.01–5 mM). The percentage of cells in repair increased in a concentration-dependent manner to a maximum of 57% at 1 mM. At the highest concentration tested (5 mM), the NNG/cell score was 13.6 ± 0.5 whereas it was -2.7 ± 0.5 for the negative control. These data, based on various endpoints, show a midly genotoxic effect of 2,4-DAT in the HepG2 cells and confirm that this cell line is a suitable model to study the toxic effects of aromatic amines.

Keywords: 2,4-Diaminotoluene; Uridine uptake assay; Comet assay; Micronucleus test; UDS assay; HepG2 cells

1. Introduction

2,4-Diaminotoluene (2,4-diamino-1-methylbenzene, 2,4-DAT) belongs to the aromatic amines group (Fig. 1); it is an industrial, chemical and an environmental pollutant. It is currently used in the production of approximately 60 different dyes, 28 of which are probably produced in significant amounts worldwide. These dyes are used to color silk, wool, paper, leather

Abbreviations: B[*a*]P, benzo[*a*]pyrene; BNC, binucleated cells; 2,4-DAT, 2,4-diaminotoluene; DMSO, dimethylsulfoxide; MN, micronuclei; NDI, nuclear division index; NNG, net nuclear grain; OTM, olive tail moment; SCGE, single-cell gel electrophoresis (syn: comet assay); TCA, trichloroacetic acid; UDS, unscheduled DNA synthesis

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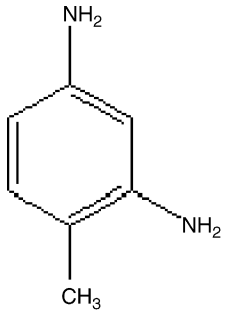

Fig. 1. Chemical structure of 2,4-diaminotoluene (2,4-DAT).

and furs (IARC, 1978). Other applications include its use for the preparation of impact resins, polyamides with superior wire coating properties, antioxidants, hydraulic fluids, polyurethane foams, fungicide stabilizers and as a photographic developer (HSDB, 2000). The primary routes of potential human exposure to 2,4-DAT are skin contact and inhalation. The National Occupational Exposure Survey (1981–1983) indicated that 8513 workers, including 395 women, were potentially exposed to 2,4-DAT (NIOSH, 1990). Consumer exposure is also possible as a result of the presence of trace contaminants in products that contain 2,4-DAT-based dyes (e.g. furs, leather, silk, textiles and wool) (IARC, 1978). Furthermore, the compound is a degradation product of the polyester urethane foam used in Meme silicone breast implants (Luu et al., 1998) and high levels (0.4–6 ng/ml) have been detected in urine and plasma from patients up to 2 years after implantation (Sepai et al., 1995; Luu et al., 1998). 2,4-DAT has been shown to be genotoxic in presence of a rat liver supernatant fraction in the *Salmonella typhimurium* assay (Ames et al., 1975) and also a potent rodent hepatocarcinogen (Ito et al., 1969). When administered in the diet, 0.1 or 0.06% 2,4-DAT induced hepatocellular carcinomas or neoplastic nodules in male and female rats (Ito et al., 1969) and fibromas of the subcutaneous tissue in male rats, it also increased the incidence of carcinomas or adenomas of the mammary gland in female rats. In mice, 2,4-DAT induced hepatocellular carcinomas and may have increased the incidence of lymphomas, both in females, but had no effect on tumor incidence in males (NCI, 1979). Injected subcutaneously, the compound induced local sarcomas in rats of both sexes (IARC, 1978, 1986). 2,4-DAT and its structural isomer 2,6-diaminotoluene have been tested for carcinogenicity in rat and mice within the National Toxicology Programme (US NTP). 2,4-DAT was found to induce liver, mammary and s.c. tumors in rats and liver tumors and lymphomas in female mice, whereas 2,6-DAT gave negative results (Anon, 1979, US NTP). The National Cancer Institute (NCI) and the International Agency of Research on Cancer (IARC) conclude that 2,4-DAT is likely to be a human carcinogen, as there is sufficient evidence of carcinogenicity in experimental animals (NCI, 1979; IARC, 1978, 1986). However, no information about the mechanisms underlying the genotoxicity of this compound is available.

2,4-DAT is metabolically converted to genotoxic intermediates by stimulating its own activation through CYP1A induction (Cheung et al., 1996). The liver is the primary target tissue for the activation of aromatic amines and therefore investigations of the biological activities of the products should be performed with hepatic cells. HepG2 cells express various phase I and phase II enzymes and reflect the metabolism of DNA-damaging compounds in mammals better than other in vitro models which require addition of exogenous mixtures. The HepG2 cell line has been successfully used for detection of genotoxic carcinogens, including compounds which give false negative results in other in vitro assays (Knasmüller et al., 1998; Uhl et al., 2000; Lu et al., 2002). Therefore, genotoxicity assays with HepG2 cells reveal hazards caused by agents potentially genotoxic to humans better than in vitro tests with bacteria and metabolically incompetent mammalian cells. We therefore used the human-derived hepatoma cell line HepG2 to investigate the genotoxicity of 2,4-DAT. We used three genotoxicity assays to study the mechanisms by which 2,4-DAT acts: the micronucleus (MN) assay; single-cell gel electrophoresis (SCGE or comet assay) and the unscheduled DNA synthesis (UDS) test. The SCGE assay is a very sensitive assay (Tice et al., 2000; Mckelvey-martin et al., 1993) based on the migration of denatured DNA fragments during electrophoresis (Östling and Johanson, 1984). Electrophoresis can be carried out under highly alkaline conditions (pH > 12.6) to detect single- and double-strand breaks and alkali-labile lesions (Singh et al., 1988). It is rapid and reliable for the quantification of DNA lesions and has been used with various cell lines (CHO, V79 and HepG2) to detect the genotoxic effects of various xenobiotics (Tice et al., 2000). UDS detection is highly suitable for