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Cytotoxic and genotoxic effects of two hair dyes used in the formulation of black color

Yaliana Tafurt-Cardona, Paula Suares-Rocha, Thaís Cristina Casimiro Fernandes, Maria Aparecida Marin-Morales*

UNESP- São Paulo State University "Júlio de Mesquita Filho" Institut of Biosciences, Department of Biology, Rio Claro Campus, Av. 24-A, 1515, Bela Vista, Rio Claro, São Paulo, 13506-900, Brazil

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ABSTRACT

According to the International Agency for Research on Cancer (IARC), some hair dyes are considered mutagenic and carcinogenic in *in vitro* assays and exposed human populations. Epidemiological studies indicate that hairdressers occupationally exposed to hair dyes have a higher risk of developing bladder cancer. In Brazil, 26% of the adults use hair dye. In this study, we investigated the toxic effects of two hair dyes, Basic Red 51 (BR51) and Basic Brown 17 (BB17), which are temporary dyes of the azo group (R–N=N–R′), used in the composition of the black hair dye. To this end, MTT and trypan blue assays (cytotoxicity), comet and micronucleus assay (genotoxicity) were applied, with HepG2 cells. For cytotoxic assessment, dyes were tested in serial dilutions, being the highest concentrations those used in the commercial formula for hair dyes. For genotoxic assessment concentrations were selected according to cell viability. Results showed that both dyes induced significant cytotoxic and genotoxic effects in the cells, in concentrations much lower than those used in the commercial formula. Genotoxic effects could be related to the azo structure present in the composition of the dyes, which is known as mutagenic and carcinogenic. These results point to the hazard of the hair dye exposure to human health.

1. Introduction

In the last decades, studies have shown that topical products can be absorbed, leading to systemic effects in the organisms exposed to them. Thereby, the possible toxic effects stemming from population exposure to cosmetic products have been reassessed (Nohynek et al., 2010). According to the International Agency for Research on Cancer (IARC), *in vitro* and *in vivo* studies (in exposed human populations) have shown that some hair dyes and many chemicals used in the hair dyeing process can be considered mutagenic and carcinogenic (IARC, 1993; IARC, 2010).

The permanent dyes represent approximately three quarters of the global dye use (Bolduc and Shapiro, 2001). At the end of the 1990s, approximately 33% of women over 18 years old and 10% of men over 40 years old in the United States used some kind of hair dye (La Vecchia and Tavani, 1995; Ghosh and Sinha, 2008). Studies in the first decade of this century showed an increase in this percentage, with 42% of the women and 25% of the men making use of hair dyes (Rosenkranz et al., 2007). In Brazil, according to the National Institute of Metrology, Standardization and Industrial Quality (INMETRO), 26% of the adults use hair dye, most of which are women (INMETRO, 2014).

In North America and Europe, there are approximately two million professional hairdressers, barbers and beauticians who are routinely exposed to hair dyes (Gago-Dominguez et al., 2001; Czene et al., 2003). Epidemiological studies indicate that hairdressers occupationally exposed to hair dyes have a higher risk of developing bladder cancer, proportional to the exposure time (<10 years, risk increased by 0.5 times; 10 years or more, risk increased by 5.1 times), and of lymphoid malignancies (Holly et al., 1998; Zhang et al., 2004). Population studies also indicate that women using permanent hair dye at least once a month have a 2.1 times higher risk of bladder cancer in comparison with those who does not use it (Gago-Dominguez et al., 2001; Letašiová et al., 2012). Andrew et al. (2004) detected the presence of components of hair dye or their derivatives in the urine of users, indicating that the carcinogenic compounds can reach the target organ, the bladder. These results may be linked to the aromatic amines present in

* Corresponding author.
E-mail address: mamm@rc.unesp.br (M.A. Marin-Morales).

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the hair dye (Yu et al., 2002). These compounds can be absorbed through the skin during the product application and induce this type of cancer which, according to *in vitro* and *in vivo* tests, are potentially mutagenic and carcinogenic (Ames et al., 1975; Bolt and Golka, 2007; Platzek, 2010).

The black hair dye is composed of a mixture of several dyes, including the pure dyes Basic Red 51 (BR51) and Basic Brown 17 (BB17), which are temporary dyes of the azo group (R–N=N–R′) (Fig. 1), and the mixture Ebony, composed of other five hair dyes. Studies performed by the Scientific Committee on Consumer Safety – SCCS/1436/11 (2011) indicated the mutagenic action of BR51 and BB17 using the Ames test with *Salmonella tiphymurim* in the presence of metabolic activation (S9) (SCCS/1436/11; SCCS/1431/14). These azo dyes have been widely used by hair dye industries. During 2005, the total production of BR51 was 0.1 and 0.5 Tons, and of BR17, 1 and 5 Tons (IARC, 2010).

Some hair dyes contain aromatic amines which may be toxic (Takkouche et al., 2005), mutagenic (Ames et al., 1975) or carcinogenic (Sontag, 1981), representing a risk to human health (Sanjosé et al., 2006), due to their worldwide use. By the widespread use of hair dyes, the proven toxicity of these chemicals and the hazard associated with their continued use, it is essential to perform further investigations of these cosmetic products to reach new information that can assist the comprehension of possible damages that these dyes can promote on the health of exposed organisms. This warning has also been suggested by the Scientific Committee on Cosmetic and Non-Food Products Intended for Consumers (SCCNFP) that, based on the available scientific information, recommends the application of different strategies for the identification of possible carcinogenic risks to humans, associated to the presence of several compounds of different kinds of dyes, including the precursors, couplers, oxidants/intermediates and the products of combined reaction (SCNFP/0720/03). This way, the present study aimed at investigating the cytotoxic and genotoxic potential of the hair dyes Basic Red 51 (BR51) and Basic Brown 17 (BB17), used in the composition of the black hair dye, using permanent cells derived from human hepatoma (HepG2). Therefore, two acute cytotoxicity assays were performed, the Thiazolyl Blue Tetrazolium Bromide (MTT) assay and the Trypan blue test (TB), which evaluate the integrity of two different cellular organelles (mitochondria and cell membranes, respectively). Moreover, in order to investigate the genotoxic potential of these dyes, comet assay and micronucleus test were also performed. The single cell gel electrophoresis assay detects single-strand breaks (SSB), double-strand breaks (DSB), alkali-labile sites (ALS) and SSB associated with incomplete repair of excision sites (Speit and Hartmann, 1995; Tice et al., 2000), while cytokinesis-block MN assay (CBMN) in the formation of micronucleus (MN), can occur for any lost or chromosomal breakage and dysfunction of the mitotic spindle, indicating important genomic instability events (Fenech, 2007; Kirsch-Volders et al., 2011). This is the first study investigating the cytotoxic and genotoxic potential of these dyes in HepG2 cells.

2. Materials and methods

2.1. Test compounds – BR51 and BB17

The compounds used in this study were the hair dyes BR51 (2-[((4-Dimethylamino)phenyl)azo])-1,3-dimethyl-1H-imidazolium chloride (CAS No. 77061-58-6) and BB17 (8-[(4-Amino-3-nitrophenyl)azo])azo]-7-hydroxy-N,N,N-trimethyl-2-naphthalenaminium chloride (CAS No. 68391-32-2) (Fig. 1), used in the formulation of black dye, according to the proportion of the commercial use, indicated by the manufacturer (Arianor Cherry Red 0.002% and Arianor Sienna Brown 0.04%). All substances were obtained in powder by the commercial dye company *Sensient Cosmetic Technologies Brasil*, from São Paulo, Brazil.

2.2. Treatment solution

The powders of each dye were dissolved in sterilized bi-distilled water, in a concentration of 20 mg/mL for BR51, and of 40 mg/mL for BB17. Then, these solutions were again re-dissolved in Minimal Essential Medium (MEM Gibco/Cultilab) and the following concentrations were tested for cell viability tests (MTT and Trypan Blue assays): BR51 2000 μg/mL, 200 μg/mL, 20 μg/mL, 2 μg/mL, 0.2 μg/mL and 0.02 μg/mL; BB17 62.5 μg/mL, 31.2 μg/mL, 15.6 μg/mL, 7.8 μg/mL, 3.9 μg/mL and 1.95 μg/mL, being the highest concentrations, the same concentrations use in the commercial formula for the hair dye. Then, the viability tests indicate the higher concentration inducing at least 80% of viability, to be use in the specific tests, where cytotoxic effects should be avoided.

2.3. Biological materials: human cell culture

HepG2 cells, isolated from human hepatoma, were used as biological material for testing the toxicity of the dyes. These cells are considered an important tool for the assessment of mutagens and pro-mutagens, since they can express different xenobiotic metabolizing enzymes (Westerink and Schoonen, 2007; Valentin-Severin et al., 2003).

HepG2 cells were obtained from the American Type Culture Collection (ATCC No HB 8065, Rockville, MD) and were cultivated in 25 cm^2 culture flasks with 5 mL of MEM (Gibco/Cultilab) supplemented with 10% fetal bovine serum (FBS) and 0.1% of antibiotic-antimycotic solution (penicillin 10,000 IU/mL/streptomycin 10 mg/mL, Cultilab). The flasks were maintained in a CO_2 incubator (5%) until reaching approximately 80% confluence with a cell cycle of approximately 24 h (Salvadori et al., 1993).

2.4. Thiazolyl Blue Tetrazolium Bromide test (MTT)

The MTT test is a cytotoxicity assay that has been used to evaluate the survival, proliferation and activation of cells. This test is

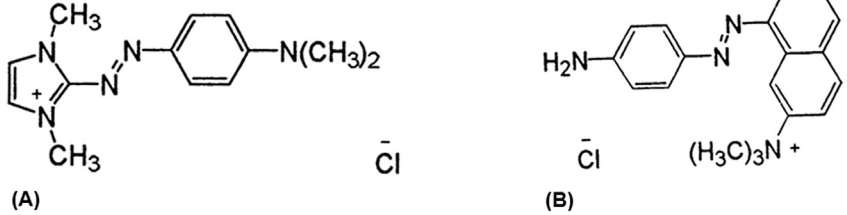

Fig. 1. Chemical structures of the dyes: (A) Basic Red 51 – BR51 (2-[((4-Dimethylamino)phenyl)azo])-1,3-dimethyl-1H-imidazolium chlorid (SCCS/1436/11); (B) Basic Brown 17 – BB17 (8-[(4-Amino-3-nitrophenyl)azo]) azo]-7-hydroxy-N,N,N-trimethyl-2-naphthalenaminium (SCCS/1431/14).