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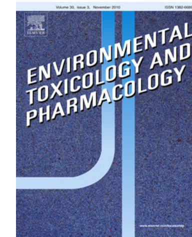

Evaluation of the *in vitro* cytogenotoxicity profile of antipsychotic drug haloperidol using human peripheral blood lymphocytes

*Goran Gajski, Marko Gerić, Vera Garaj-Vrhovac**

Institute for Medical Research and Occupational Health, Mutagenesis Unit, 10000 Zagreb, Croatia

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ABSTRACT

Haloperidol (HLP) is a potent antipsychotic drug that is commonly used for the treatments of schizophrenia and bipolar disorders but has a tendency to cause adverse effects. In the present study, the cyto/genotoxic potential of clinically relevant concentrations of HLP was evaluated in human peripheral blood lymphocytes (HPBLs) as sensitive biomarkers of exposure. HLP was administered as HLP hydrochloride in the final concentrations of 5, 10 and 20 ng/ml for 4 and 24 h period. Cytotoxicity was determined using differential staining of HPBLs with acridine orange and ethidium bromide while chromosomal aberrations, micronucleus and comet assays were applied to estimate the chromosomal and DNA damage after the treatment. The results of the present study indicate that HLP is capable of inducing cyto/genotoxicity in tested cells. Present study has also confirmed the need for further cytogenetic research and regular patient monitoring to minimize the risk of any possible adverse events.

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1. Introduction

Antipsychotics are drugs of long-term use that are administered to a large number of patients. Haloperidol (HLP) is a commonly used butyrophenone derivative with antipsychotic activity (Beresford and Ward, 1987). The chemical name of HLP (Fig. 1) is 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone. It is light yellow crystalline powder, have melting point 149–153 °C, pH 2.8–3.6 and pK_a of 8.3. It is soluble in solvents like glacial acetic acid, chloroform, ethanol and dimethyl sulfoxide (DMSO) but almost insoluble in water. As a dopamine antagonist with selectivity for D_2-like receptors it is used for the symptomatic management of psychotic disorders. Drug therapy is important to the management of acute psychotic episodes and accompanying violent behaviour in patients with schizophrenia, generally required for long-term stabilization to improve symptoms between episodes and to

Abbreviations: AO, acridine orange; CA, chromosomal aberrations; CBMN, cytokinesis-block micronucleus assay; DMSO, dimethyl sulfoxide; EtBr, ethidium bromide; GSH, glutathione; GPx, glutathione peroxidise; HLP, haloperidol; HPBL, human peripheral blood lymphocyte; LMP, low melting point; LPO, lipid peroxidation; LTN, long-tailed nuclei; MDA, malondialdehyde; MN, micronucleus; MNi, micronuclei; NBUD, nuclear bud; NDI, nuclear division index; NMP, normal melting point; NPB, nucleoplasmic bridge; ROS, reactive oxidative species; SCGE, single cell gel electrophoresis assay; SOD, super oxide dismutase; TBARS, thiobarbituric acid reactive species.

* *Corresponding author at:* Institute for Medical Research and Occupational Health, Mutagenesis Unit, Ksaverska cesta 2, 10000 Zagreb, Croatia. Tel.: +385 1 4682 500; fax: +385 1 4673 303.

E-mail addresses: ggajski@imi.hr (G. Gajski), vgaraj@imi.hr (V. Garaj-Vrhovac).

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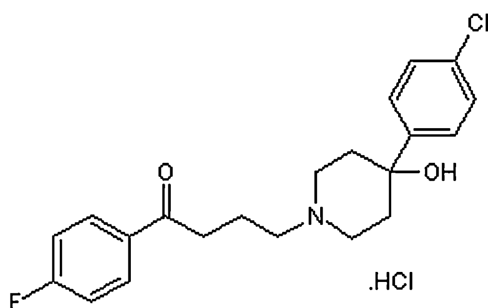

Fig. 1 – Chemical structure of haloperidol hydrochloride.

minimize the risk of recurrent acute episodes (Gilman et al., 1990; Jain et al., 2011; Katzung, 2001; Powney et al., 2012).

Antipsychotic drugs that include HLP might be expected to give rise to acute extrapyramidal symptoms, tardive dyskinesia and symptoms related to hyperprolactinaemia (Adams et al., 2001; Haddad et al., 2009). HLP is also associated with a dose-dependent rise in plasma prolactin and in one study caused extrapyramidal symptoms severe enough to warrant anti-Parkinsonian drugs in around 90% of patients (Chouinard et al., 1989). Although HLP is often assumed to give the highest risk of extrapyramidal symptoms, this has not been demonstrated in comparative studies (Kissling et al., 1985; Wistedt et al., 1984, 1991). Weight gain may be less common than with fluphenazine decanoate (Taylor, 2009; Wistedt, 1986). In the evaluation of the benefit/risk ratio it should be considered that among the various adverse reactions that these drugs might cause the occurrence of a genotoxic and carcinogenic effect cannot be excluded as well (Brambilla et al., 2009).

Up till now very few studies have been reported on cyto/genotoxicity of HLP in normal human cells *in vitro*. In this kind of assessment combination of different methods may play an important role in the assessment of cyto/genotoxic damage caused by this type of drugs and these methods make it possible to evaluate the level of primary DNA damage or the dynamics of its repair even after short-term exposure to cyto/genotoxic agents (Abou-Eisha and Afifi, 2004). Considering that, and the lack of data on the cytogenetic status induced by HLP, the aim of this study was to evaluate the cyto/genotoxic potential of clinically relevant concentrations of HLP *in vitro* in human peripheral blood lymphocytes (HPBLs) as sensitive biomarkers of exposure using a battery of bioassays.

2. Materials and methods

2.1. *Chemicals*

HLP hydrochloride was from Tocris Bioscience (Bristol, UK); Chromosome kit P was from Euroclone (Milano, Italy); RPMI 1640 medium was from Invitrogen (Carlsbad, CA, USA); acridine orange (AO), colchicine, cytochalasin-B, histopaque, ethidium bromide (EtBr), low melting point (LMP) and normal melting point (NMP) agaroses were from Sigma (St Louis, MO, USA); heparinised vacutainer tubes were from Becton Dickinson (Franklin Lakes, NJ, USA); Giemsa dye was from Merk (Darmstadt, Germany). All other reagents used were laboratory-grade chemicals from Kemika.

2.2. *Blood sampling and treatment*

Evaluation of HLP was performed on HPBLs obtained from a young, healthy, non-smoking, female donor. The donor had not been exposed to ionizing radiation for diagnostic or therapeutic purposes or to known genotoxic chemicals that might have interfered with the results of the testing in the 12-month period prior to the blood sampling. The subject gave informed consent to participate in this study. The study was a part of the project approved by the institutional ethics committee and observed the ethical principles of the Declaration of Helsinki. Blood was drawn by antecubital venipuncture into heparinized vacutainers containing lithium heparin as anticoagulant under aseptic conditions.

Just before the beginning of the experiment, HLP hydrochloride was dissolved in DMSO. All experiments were conducted on the same blood sample treated with HLP in the final concentrations of 5, 10 and 20 ng/ml for 4 and 24 h. The concentrations used were those found in plasma samples of schizophrenic patients (Jain et al., 2011). The *in vitro* treatment in the present study was performed on non-dividing HPBLs (G_0). Blood samples were incubated *in vitro* at 37 °C in a humified atmosphere with 5% CO_2 (Heraeus Heracell 240 incubator, Langenselbold, Germany).

2.3. *Cell viability (cytotoxicity) test*

The indices of cell viability and necrosis were established by differential staining of HPBLs with AO and EtBr using fluorescence microscopy (Duke and Cohen, 1992). Lymphocytes were isolated using a modified Ficoll–Histopaque centrifugation method (Singh, 2000). The slides were prepared using 200 μl of HPBLs and 2 μl of stain (AO and EtBr). The suspension mixed with dye was covered with a cover slip and analyzed under an epifluorescence microscope Olympus BX51 (Tokyo, Japan) at 400× magnification. A total of 100 cells per repetition were examined. The nuclei of vital cells emitted a green fluorescence and necrotic red fluorescence.

2.4. *Chromosome aberrations (CA) test*

The CA test was performed according to current IAEA Guidelines (2001). After the exposure to HLP the whole blood (500 μl) was incubated in a Euroclone medium at 37 °C for 48 h in an atmosphere of 5% CO_2. To arrest dividing lymphocytes in metaphase, colchicine (0.004%) was added 3 h prior to the harvest. Cultures were centrifuged at 1000 rpm for 10 min, the supernatant was carefully removed, and the cells were resuspended in a hypotonic solution (0.075 M KCl) at 37 °C. After centrifugation for 10 min at 1000 rpm, the cells were fixed with a freshly prepared fixative of ice cold methanol/glacial acetic acid (3:1, v/v). Fixation and centrifugation were repeated several times until the supernatants were clear. Cells were pelleted and resuspended in a minimal amount of fresh fixative to obtain a homogeneous suspension. The cell suspension was dropped onto microscope slides and left to air-dry. Slides were stained with 5% Giemsa solution. All slides were

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