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Assessment of the genotoxic potential of the antipsychotic sigma receptor ligand E-5842

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Abstract

The genotoxic potential of E-5842, a sigma ligand compound being developed as an antipsychotic drug, was evaluated by means of an extensive battery of in vitro and in vivo assays. Negative results were obtained in an Ames test (up to 5000 μg/plate), a mouse lymphoma assay (up to 535.1 μg/ml (−S9) and 891.8 μg/ml (+S9)), an in vivo rat hepatocyte micronucleus assay (up to 100 mg/kg/day on 2 days), and a two-dose mouse micronucleus assay (up to 40 mg/kg/day on 2 days). In a single-dose mouse bone-marrow micronucleus assay (up to 400 mg/kg; 24, 48 and 72 h sampling) a slight and non-statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (MNPCE) was observed 48 h after administration of a 200 mg/kg dose, in the absence of bone-marrow toxicity. This minor increase in MNPCE frequency was considered of questionable biological relevance, because it was observed under conditions of marked animal toxicity including mortality. In addition, it occurred in association with a strong hypothermic effect produced by administration of E-5842.

A clear increase in the frequency of structural chromosomal aberrations was observed in human lymphocytes at concentrations ≥350.6 and 1685.4 μg/ml in the presence and absence of S9, respectively. Mitotic accumulation was observed at those concentrations at which clastogenic effects were observed, a condition that may have masked toxicity. Concentrations lacking clastogenic effects in this chromosome aberration assay (300.7 and 173.2 μg/ml in the presence and absence of S9, respectively) were well in excess of maximum human plasma concentrations attained in clinical studies at the maximum tolerated dose (19.1 ng/ml). A weight-of-evidence analysis, taking into consideration the results obtained in the different in vitro and in vivo assays and the conditions of clinical use, suggest that E-5842 would not pose a genotoxic risk under clinical conditions.
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Keywords: Sigma receptor; Genotoxicity; Antipsychotic drug; Risk assessment; Mitotic accumulation

1. Introduction

Schizophrenia is a serious central nervous system disorder of still unknown aetiology, characterized by disturbances in form and content of thought, mood behaviour, sense of self and relationship to the external world. The symptoms of this disorder have been classified according to positive and negative symptoms. Positive symptoms include hallucinations, delusions, psychosis, paranoia, disorganized speech and behaviour, and negative symptoms include loss of energy, deficiency of speech, lack of initiative, loss of sociability and blunting of emotions [1]. Dopamine D_2 receptor antag-

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onists (D_2 antagonists), the major therapeutic agents for treatment of schizophrenia, have been shown to be effective in improving positive symptoms of schizophrenia. However, D_2 antagonists are not sufficiently effective in improving the negative symptoms of schizophrenia and they induce clinical extrapyramidal adverse effects (e.g. dystonia, tardive dyskinesia, and akathisia). Therefore, there is a need to search for novel antipsychotic agents with different mechanism(s) of action and better activity and tolerability profiles [2]. Among these new agents, sigma (σ) receptor ligands have emerged as potential pharmacological targets for developing compounds with antipsychotic properties. The hypothesis for the possible use of σ ligands for treatment of schizophrenia originated from the observation that some of the typical antipsychotic agents such as haloperidol, perphenazine and chlorpromazine, which are D_2 antagonists, also have affinities for σ receptors [3]. Antipsychotic properties have been demonstrated for σ receptor ligands in both animal studies and clinical trials [4,5].

E-5842 (4-(4-fluorophenyl)-1,2,3,6-tetrahydro-1-[4-(1,2,4-triazol-1-il)butyl]pyridine citrate, CAS-220120-14-9) is a newly developed and potential atypical antipsychotic drug with very high affinity for the σ_1 receptor ($K_i = 4$ nM), and moderate to low affinity for other central nervous system receptors, including the dopamine D_1, D_2, D_3, and D_4 receptors and the serotonin 5-HT_2 and 5-HT_{1A} receptors [6]. E-5842 has shown to be active in different animal models predictive of antipsychotic activity, with a low tendency to cause extrapyramidal side-effects and with efficacy against positive and negative symptoms of schizophrenia [6]. Assessment of genotoxic potential is a crucial and mandatory step in the development process of a pharmaceutical compound, because of the nature of the effect being evaluated and its relevance to human safety in relation to the potential induction of cancer and hereditary defects. Here we report the results of the battery of studies that were undertaken to evaluate the possible genotoxic potential of E-5842, as part of the preclinical safety-assessment program for development of this substance as a putative antipsychotic compound. E-5842 was tested for genotoxicity in a battery of three in vitro and three in vivo assays: an Ames test, an in vitro chromosome aberration assay in human lymphocytes, an in vitro gene mutation assay in mouse lymphoma L5178Y $tk^{+/-}$ cells, an in vivo rat hepatocyte micronucleus assay, and both a single-dose and a two-dose in vivo mouse micronucleus assay. The results obtained in these studies and their relevance to risk assessment are discussed.

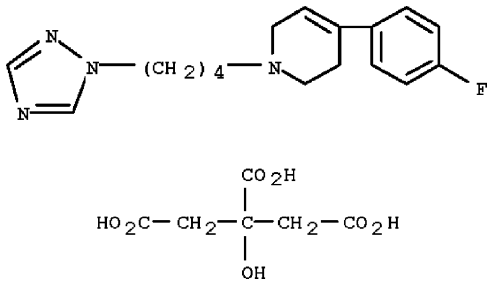

Fig. 1. Chemical structure of E-5842.

2. Materials and methods

2.1. Test substance

E-5842 bulk powder was synthesised by Laboratorios Dr. Esteve (Barcelona, Spain) (Fig. 1). E-5842 was administered as its citrate salt in all studies except for the mouse lymphoma assay where it was administered as its hydrochloride salt. Purity was always above 98.5%. For exposure comparison with animal and human plasma levels, in vitro tested concentrations of E-5842 are expressed as equivalents of its base. Test article solutions were prepared by dissolving E-5842 in reverse-osmosis water immediately prior to the assays. For in vitro studies, a stock solution was prepared at the maximum required treatment concentration, filter-sterilized (0.22 μm) and then further diluted in sterile reverse-osmosis water to the required concentrations. Analysis of the concentration of the test substance was performed in one of the in vitro assays and confirmed the validity of the formulation procedure. The vehicle (reverse-osmosis water) was used as negative control for all studies. Positive control chemicals were obtained from the following manufacturers: cyclophosphamide (CP, Sigma), 4-nitroquinoline 1-oxide (NQO, Aldrich and Fluka), 2-nitrofluorene (2NF, Koch-Light), sodium azide (NaN_3, Sigma), 9-aminoacridine (AAC, Koch-Light), 2-aminoanthracene (AAN, Sigma), benzo(*a*)pyrene (BP, Aldrich), dimethylnitrosamine (DMN, Sigma). All other chemicals were of the purest grade available. Prior to the assays, NaN_3 and DMN were dissolved in water, CP was dissolved in water or physiological saline (for the single-dose and the two-dose mouse micronucleus assays, respectively), and the remaining positive control chemicals were dissolved in DMSO.

2.2. Metabolic activation system

For all in vitro assays, mammalian liver post-mitochondrial faction (S9) was used for metabolic activation. S9 fraction prepared from the livers of male Sprague–Dawley rats induced with Aroclor 1254 was obtained from Molecular Toxicology Incorporated (Moltox; Maryland, USA), stored frozen at approximately −80 °C, thawed just prior to use and supplemented with NADPH-generating system. Final concentrations

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