Mutation Research 814 (2017) 14–21

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Assessment of the genotoxicity of the tyrosine kinase inhibitor imatinib mesylate in cultured fish and human cells

Matjaž Novak [a,b,c], Bojana Žegura [a], Jana Nunić [a], Goran Gajski [d], Marko Gerić [d], Vera Garaj-Vrhovac [d], Metka Filipič [a,*]

[a] Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
[b] Ecological Engineering Institute, Ljubljanska ulica 9, 2000 Maribor, Slovenia
[c] Jozef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia
[d] Mutagenesis Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form
17 December 2016
Accepted 19 December 2016
Available online 24 December 2016

Keywords:
Imatinib mesylate
Cytotoxic
Genotoxic
Zebrafish liver cells
HepG2 cells
Human peripheral blood lymphocytes

ABSTRACT

The selective tyrosine kinase inhibitor imatinib mesylate (IM) is a widely used anticancer drug. Recent studies showing that IM can induce DNA and chromosomal damage in crustaceans and higher plants prompted us to re-examine its potential genotoxicity. IM was not mutagenic in the Ames assay (*Salmonella typhimurium*). Cytotoxicity and genotoxicity were evaluated *in vitro* in zebrafish (*Danio rerio*) liver (ZFL), human hepatoma (HepG2), and human peripheral blood lymphocyte (HPBL) cells. Genotoxicity was determined with the comet assay and with the cytokinesis-block micronucleus cytome assay. ZFL and HPBL cells showed comparable sensitivity to IM cytotoxicity, while HepG2 cells were less sensitive. At non-cytotoxic concentrations, IM induced DNA strand breaks in ZFL and HepG2 cells. An increase in the number of micronuclei was observed in ZFL and HPBL cells. In HPBLs, IM also induced an increase in the number of nucleoplasmic bridges and nuclear buds. Based on the data of the consumption of IM in European countries the predicted environmental concentrations (PEC) were calculated to be in the range between 3.3 and 5.0 ng/L, which are several orders of magnitude lower from those that caused adverse effects in fish and human derived cells. However, based on the *in vitro* studies it is not possible to quantitatively predict the hazard for wildlife and humans, therefore further studies are warranted to explore the underlying molecular mechanisms of induced IM genotoxic effects as well as the studies of the occurrence of IM in the aquatic and occupational environment to establish the relevance of these observations for aquatic organisms and occupationally exposed personnel.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Imatinib mesylate (IM, Fig. 1) was the first protein kinase inhibitor used for targeted cancer chemotherapy. In contrast to cytotoxic chemotherapy drugs, protein kinase inhibitors do not target DNA. As such, they constitute a new and promising approach to cancer therapy, with high specificity to tumour cells and less toxicity to normal cells [1]. IM was developed in the mid-1990s for the treatment of BCR-ABL-associated chronic myelogenous leukaemia; it entered the market in 2001 [2] and led to a major improvement in therapeutic outcome. The mechanism of action of IM is selective inhibition of specific tyrosine kinases, including the chimeric BCR-ABL fusion oncoprotein and the transmembrane receptors KIT and PDGFR [3,4]. The Food and Drug Administration (FDA), USA, has approved IM for therapy of multiple haematological malignancies including Philadelphia chromosome-positive acute lymphocytic leukaemia and chronic myeloid leukaemia, gastrointestinal stromal tumours (KIT^+), and dermatofibrosarcoma protuberans.

IM is a widely used anticancer drug [5,6]. Some of the preclinical toxicological investigations required for its approval were reported by Cohen et al. [2] and in the assessment reports available from the European Medicines Agency [7,9] and FDA [8]. Genotoxicity studies demonstrated that IM is not mutagenic in the Ames test or mouse lymphoma assays. IM was clastogenic in the Chinese hamster ovary cell assay, but only at the highest concentration tested (125 μg/mL) in the presence of metabolic activation [2,7]. In the rat bone marrow micronucleus (MN) assay *in vivo*, IM was negative [7,8]. Two manufacturing intermediates, which may be present as trace impu-

* Corresponding author.
E-mail addresses: matjaz.novak@nib.si (M. Novak), bojana.zegura@nib.si (B. Žegura), jana1rs@yahoo.com (J. Nunić), ggajski@imi.hr (G. Gajski), mgeric@imi.hr (M. Gerić), vgaraj@imi.hr (V. Garaj-Vrhovac), metka.filipic@nib.si (M. Filipič).

http://dx.doi.org/10.1016/j.mrgentox.2016.12.002
1383-5718/© 2016 Elsevier B.V. All rights reserved.

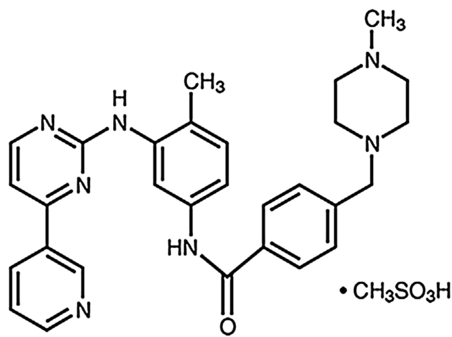

Fig 1. Structure of imatinib mesylate.

rities in the final product, are mutagenic in the Ames test; one of these is also positive in the mouse lymphoma assay, but negative in the rat bone marrow MN assay [8,9]. Taking into account the negative results of the *in vivo* studies, and in the context of its therapeutic indication and effectiveness, concern with regard to IM genotoxicity has not been high [7].

Recent studies showed that IM induce DNA damage in crustaceans [10], associated with inhibition of reproduction [11]. IM also inhibited reproduction of algae and cyanobacteria [12]. In higher plants, IM induced MN formation in *Tradescantia* and *Allium cepa* and affected the fertility of plant species such as *Chelidonium majus*, *Tradescantia palludosa*, and *Arabidopsis thaliana* [13–15].

The aim of this study was to assess the *in vitro* cytotoxicity and genotoxicity of IM in cells that do not express the target aberrant tyrosine kinases: zebrafish (*Danio rerio*) liver (ZFL) cells; human hepatoma (HepG2) cells; and primary human peripheral blood lymphocytes (HPBL). ZFL cells are considered to be relevant for identification of toxicological hazards to aquatic organisms. HepG2 and HPBL cells are non-target cells of human origin that are commonly used in cytotoxicity and genotoxicity studies *in vitro* for toxicological hazard identification [16]. Genotoxicity was determined using the comet assay, which measures the induction of DNA strand breaks [17], and the cytokinesis-block micronucleus cytome (CBMN) assay, which measures the induction of genomic instability [18]. Additionally, the mutagenicity of IM was determined using the *Salmonella typhimurium* reverse mutation assay (Ames assay).

2. Materials and methods

2.1. Chemicals

IM (Lot Number A1712; purity > 99%) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A stock solution of IM (50 mg/mL; 85 mM) was prepared in sterile double-distilled water purified with the Milli-Q system (Millipore, Bedford, USA), aliquoted, and stored at −20 °C.

Supplies were obtained as follows. Chromosome kit P, Euroclone (Milan, Italy); RPMI 1640, HEPES and epidermal growth factor, Invitrogen (Carlsbad, CA, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acridine orange (AO), bleomycin, cytohalasin-B, dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), histopaque, low-melting-point (LMO) agarose, normal-melting-point (NMP) agarose, methanol, and William's medium E, Sigma Chemicals (St Louis, MO, USA); penicillin/streptomycin, foetal bovine serum (FBS) for HepG2 cells, L-glutamine, and phosphate-buffered saline (PBS), PAA Laboratories (Dartmouth, MA, USA); trypsin, BD-Difco (Le Pont-De-Claix Cedex, France); Leibovitz L-15 medium and foetal bovine serum for ZFL cells, American Type Culture Collection (Manassas, VA, USA); Dulbecco's modified Eagle's medium and Ham's F-12 medium, Gibco (Waltham, MA, USA); heparinised vacutainer tubes, Becton Dickinson (Franklin Lakes, NJ, USA); Giemsa stain, Merck (Darmstadt, Germany); lyophilised Aroclor-1254-induced male rat-liver post-mitochondrial fraction (S9), Moltox (Boone, NC, USA). All other reagents were the purest grades available, and solutions were made using Milli-Q water.

2.2. Salmonella typhimurium/*microsomal (Ames) assay*

IM mutagenicity was tested in the plate incorporation assay with *S. typhimurium* strains TA97a, TA98, TA100, TA102, and TA1535, with or without metabolic activation (S9) [19]. The bacterial strains were obtained from Trinova Biochem GmbH (Giessen, Germany). Briefly, IM (100 μL H 0.3125, 0.625, 1.25, 2.5, and 5 mg/mL, corresponding to doses of 31.25, 62.5, 125, 250, and 500 μg/plate, respectively), overnight culture of *S. typhimurium*, 100 μL, and phosphate buffer, 500 μL (for assays without metabolic activation) or 4% S9 mix (for assays with metabolic activation) were added to 2 mL molten top agar containing a limited amount of histidine/biotin; these were mixed gently and poured onto minimal agar plates. The plates were incubated for 48 h (TA97a, TA100, TA102, TA1535) and 72 h (TA98) at 37 °C. Subsequently, the numbers of spontaneous and IM-induced His^+ revertants were counted. The plates were checked for possible toxic effects of IM. Three plates were used per experimental point.

Positive controls (direct mutagenicity) were 4-nitroquinoline-*N*-oxide (NQO), 0.25 μg per plate, for TA97a and TA98; mitomycin C, 0.05 μg/plate, for TA102; and sodium azide (NaN_3), 0.25 and 0.125 μg/plate, respectively, for TA100 and TA1535. The positive control (S9-dependent mutagenicity) was benzo[*a*]pyrene (B*a*P), 5 and 2.5 μg/plate for TA97a and for TA98, TA100, TA102 and TA1535, respectively.

2.3. Cell cultures

The ZFL cell line, derived from normal adult zebrafish [20], was obtained from American Type Culture Collection (N° CRL-2634). The cells were cultured under a humidified air atmosphere at 28 °C in a medium composed of 50% Leibovitz L-15 medium, 35% Dulbecco's modified Eagle's medium, and 15% Ham's F-12 medium, supplemented with HEPES, 15 mM; $NaHCO_3$, 0.15 g/L; insulin, 0.01 mg/mL; epidermal growth factor, 50 ng/mL; penicillin/streptomycin, 100 U/mL; and 5% heat-inactivated FBS.

HepG2 cells were a gift from Dr. Firouz Darroudi (Leiden University Medical Centre, Department of Toxicogenetics, Leiden, Netherlands). The cells were cultured under a humidified air/5% CO_2 atmosphere at 37 °C (Heraeus Heracell 240 incubator, Langenselbold, Germany) in William's medium E containing 15% FBS, L-glutamine, 2 mM, and penicillin/streptomycin, 100 U/mL.

HPBL were obtained from three young healthy male donors. The donors gave informed consent to participate in this study, which was part of a project approved by the Ethics Committee and following the ethical principles of the Declaration of Helsinki. According to the questionnaires filled out by the donors, they had not been exposed to diagnostic radiation or to known genotoxic chemicals that might have interfered with the results of the tests, for at least a year before the blood sampling. Blood was drawn by antecubital venipuncture into heparinised vacutainers containing lithium heparin as anticoagulant and under aseptic conditions. The experiments were conducted on whole blood, cultivated under a humidified air/5% CO_2 atmosphere at 37 °C.

2.4. Cytotoxicity assays

Cytotoxicity of IM to ZFL and HepG2 cells was determined by measuring their viability after exposure to IM, using the MTT assay

Download English Version:

https://daneshyari.com/en/article/5528743

Download Persian Version:

https://daneshyari.com/article/5528743

Daneshyari.com