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Combined cyto/genotoxic activity of a selected antineoplastic drug mixture in human circulating blood cells

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HIGHLIGHTS

- An antineoplastic drug mixture was tested in human blood cells.
- The mixture induced a time- and dose-dependent cyto/genotoxic effect.
- Antineoplastic drug combinations can pose risks to human health.
- Further toxicological screening of antineoplastic drug mixtures are warranted.

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ABSTRACT

Antineoplastic drugs are highly cytotoxic chemotherapeutic agents that can often interfere directly or indirectly with the cell's genome. In an environmental or medical setting simultaneous exposure may occur. Such multiple exposures may pose a higher risk than it could be assumed from the studies evaluating the effect of a single substance. Therefore, in the present study we tested the combined cyto/genotoxicity of a mixture of selected antineoplastic drugs with different mechanisms of action (5-fluorouracil, etoposide, and imatinib mesylate) towards human lymphocytes *in vitro*. The results suggest that the selected antineoplastic drug mixture is potentially cyto/genotoxic and that it can induce cell and genome damage even at low concentrations. Moreover, the changes in the measured oxidative stress parameters suggest the participation of reactive oxygen species in the cyto/genotoxicity of the selected mixture. The obtained results indicate not only that such mixtures may pose a risk to cell and genome integrity, but also that single compound toxicity data are not sufficient for the predicting toxicity in a complex environment. Altogether, the results emphasise the need for further toxicological screening of antineoplastic drug mixtures, especially at low environmentally relevant concentrations, as to avoid any possible adverse effects on the environment and human health.

1. Introduction

Antineoplastic drugs have been widely used for chemotherapy. However, many of them have been categorised as persistent carcinogenic, mutagenic and teratogenic compounds, triggering widespread concerns about their occupational exposure and eco-toxicological risks (Besse et al., 2012; Toolaram et al., 2014; Villarini et al., 2016; Zhang et al., 2013). The occurrence of antineoplastic drugs residues in the environment is, compared to many other pharmaceuticals, much lower. Their mechanisms of action (MoA) mainly target the prevention of growth and division of tumour cells via interference with the genetic material of the cells. However, antineoplastic drugs may not be selective enough to target only tumour cells but can also act on other types of dividing cells and tissues of exposed organisms (Besse et al., 2012; Deblonde and Hartemann, 2013; Kosjek and Heath, 2011; Toolaram et al., 2014; Zhang et al., 2013; Zounkova et al., 2010).

Antineoplastic drugs, their metabolites and transformation products (TPs) are detected worldwide in the aquatic environment and they usually occur at ng/L to μg/L levels or even below (Kosjek and Heath, 2011; Toolaram et al., 2014; Zhang et al., 2013). In fact, they have been unrestrictedly discharged into the environment not only through industry and sewage treatment plants but also from

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hospital effluents. The knowledge regarding their effects on humans and wild life once they have entered the environment is rather limited (Besse et al., 2012; Deblonde and Hartemann, 2013; Kosjek and Heath, 2011; Toolaram et al., 2014; Zhang et al., 2013; Zounkova et al., 2010). Antineoplastic drugs are designed to have specific biological interferences on targeted tissues in human bodies; therefore, many of them have been determined or suspected for their harmful effects even at low environmental levels (Zhang et al., 2013). Hence, the continuous life-long exposure to antineoplastic drugs, their metabolites and TPs including their possible additive and/or synergistic effects may put the ecosystem and humans to further threat if such exposure lingers.

There is a large number of antineoplastic drugs currently on the market with different MoA and their adverse effects have been well established in various test systems (Brezovšek et al., 2014; Gajski et al., 2016; Gerić et al., 2014; Novak et al., 2016; Parrella et al., 2015; Toolaram et al., 2014; Zounkova et al., 2010). However, the effects of low environmentally relevant concentrations of these drugs are still a matter of debate. The genotoxic effects of antineoplastic drugs in non-tumour cells are of special significance due to the possibility that they may induce genetic alterations in normal cells and/or secondary tumours in cancer patients. Antineoplastic drugs can interact with DNA directly or indirectly causing DNA damage and/or inhibiting DNA synthesis, as well as affecting mitosis and inhibiting cell proliferation (Toolaram et al., 2014). These actions can involve the unspecific inhibition of normal cells thus presenting a danger not only to humans but also to different environmental organisms. Besides, with increasing life expectancy and standard of living on a world scale, it has to be expected that the contribution of antineoplastic drugs to the environment will increase further.

Therefore, the aim of the present study was to investigate a possible combined cyto/genotoxic and oxidative potential of three antineoplastic drugs in a mixture; 5-fluorouracil (5-FU), etoposide (ET) and imatinibe mesylate (IM) (Table 1) in an experimental model with human peripheral blood lymphocytes (HPBLs) *in vitro*. Specific antineoplastic drugs were selected by the fact that they are among the most consumed anticancer drugs and based on their different MoA′.

5-FU is a pyrimidine analogue that belongs to the group of antimetabolites and is among the most consumed anticancer drugs (Besse et al., 2012; Longley et al., 2003; Zhang et al., 2008). It interferes with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell death (Noordhuis et al., 2004; Thomas and Zalcberg, 1998). Besides, treatment of cells with 5-FU leads to an accumulation of cells in S-phase and has been shown to induce p53 dependent apoptosis (Longley et al., 2003; Zhang et al., 2008).

ET is a topoisomerase inhibitor that causes an increase in DNA and chromosomal damage and cell death. It is used to treat a wide spectrum of human cancers and is often used in combination with other antineoplastic drugs (Hande, 1998; Meresse et al., 2004; Valkov and Sullivan, 2003). The primary cytotoxic target for ET is topoisomerase II, which regulates DNA under- and over-winding, and removes knots and tangles from the genome by generating transient double-stranded breaks (Baldwin and Osheroff, 2005).

IM is a protein kinase inhibitor developed for targeted chemotherapy. IM selectively inhibits the Bcr-Abl tyrosine kinase, and is used as the first therapeutic choice against chronic myelogenous leukaemia (Al-Hadiya et al., 2014; Hartmann et al., 2009; Moen et al., 2007). It also inhibits some other tyrosine kinase activities, which indicates its potential use for the treatment of other cancers (Palmberg et al., 2009). The consumption of protein kinase inhibitors, including IM, is increasing rapidly and is at present one of the most consumed anticancer drugs (Besse et al., 2012).

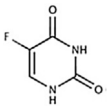
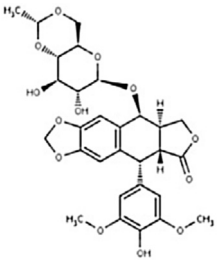
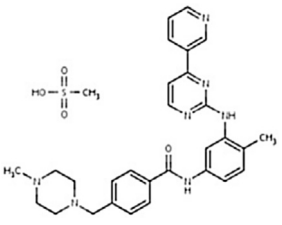
In order to evaluate the induction of DNA strand breaks and genomic instability, the comet assay (Azqueta and Collins, 2013) and its formamidopyrimidine-DNA glycosylase (Fpg)-modification for the detection of oxidative DNA damage (Collins, 2014), cytokinesis-block micronucleus (CBMN) assay (Fenech et al., 2011), and sister chromatid exchange (SCE) assay (Wilson and Thompson, 2007) were used. Moreover, the participation of oxidative stress in the cyto/genotoxicity of the selected mixture was assayed by measuring malondialdehyde (MDA) level as a measure of lipid peroxidation (LPO) (Del Rio et al., 2005) and protein carbonyls' (PC) level as a measure of oxidised proteins (OXP) (Augustyniak et al., 2015). Overall, the study provides new knowledge about the impact of antineoplastic drug mixtures on non-target, human blood cells, which is necessary for future human and environmental risk assessment.

2. Materials and methods

2.1. Chemicals

Chromosome kit P was from Euroclone (Milano, Italy); 5-bromodeoxyuridine (BrdU), 5-fluorouracil (5-FU; MW 130.08 g/mol; CAS 51-21-8), acridine-orange (AO), bleomycin, colchicine, cytohalasin-B, ethidium-bromide (EtBr), histopaque, 1,1,3,3-tetramethoxy propane (TMP), thiobarbituric acid (TBA), low

Table 1
The antineoplastic drugs investigated in the present study.

Name	5-Fluorouracil	Etoposide	Imatinib mesylate
Abbreviation	5-FU	ET	IM
CAS N°	51-21-8	33419-42-0	220127-57-1
Chemical structure			
ATC code	L01BC02	L01CB01	L01XE01
MW (g/mol)	130.08	588.57	589.71

CAS N°, Chemical Abstract Services number; ATC code, anatomical therapeutic chemical classification code; MW, molecular weight.