Environmental Research 161 (2018) 26–34

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Genotoxicity assessment of a selected cytostatic drug mixture in human lymphocytes: A study based on concentrations relevant for occupational exposure

Goran Gajski[a,*], Carina Ladeira[b,c], Marko Gerić[a], Vera Garaj-Vrhovac[a], Susana Viegas[b,d,**]

[a] Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia
[b] Grupo de Investigação em Ambiente e Saúde, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisbon, Portugal
[c] Grupo de Investigação em Genética e Metabolismo, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisbon, Portugal
[d] Centro de Investigação em Saúde Pública, Escola Nacional de Saúde Pública, Universidade NOVA de Lisboa, Lisbon, Portugal

ARTICLE INFO

Keywords:
5-fluorouracil
Cyclophosphamide
Paclitaxel
Human lymphocytes
Cyto/genotoxicity

ABSTRACT

Cytostatic drugs are highly cytotoxic agents used in cancer treatment and although their benefit is unquestionable, they have been recognized as hazardous to healthcare professionals in occupational settings. In a working environment, simultaneous exposure to cytostatics may occur creating a higher risk than that of a single substance. Hence, the present study evaluated the combined cyto/genotoxicity of a mixture of selected cytostatics with different mechanisms of action (MoA; 5-fluorouracil, cyclophosphamide and paclitaxel) towards human lymphocytes in vitro at a concentration range relevant for occupational as well as environmental exposure. The results suggest that the selected cytostatic drug mixture is potentially cyto/genotoxic and that it can induce cell and genome damage even at low concentrations. This indicates not only that such mixture may pose a risk to cell and genome integrity, but also that single compound toxicity data are not sufficient for the prediction of toxicity in a complex working environment. The presence of drugs in different amounts and with different MoA suggests the need to study the relationship between the presence of genotoxic components in the mixture and the resulting effects, taking into account the MoA of each component by itself. Therefore, this study provides new data sets necessary for scientifically-based risk assessments of cytostatic drug mixtures in occupational as well as environmental settings.

1. Introduction

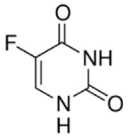
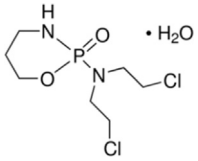
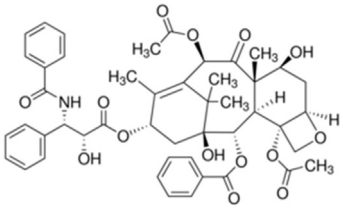
Cytostatic drugs have been recognized as hazardous to healthcare professionals since the 1970s (Falck et al., 1979). Their risk has also been recognized by several European agencies and organizations, such as the European Agency for Safety and Health at Work (EU-OSHA (European Agency for Safety and Health at Work), 2014), the Directorate-General for Employment, Social Affairs and Inclusion (EC (European Commission), 2011) as well as through national guidelines in most EU Member States (EP (European Parliament), 2016). The mechanisms of action (MoA) of these drugs are essentially related to the prevention of growth and tumour cells division via interference with a cell's genetic material. The most commonly used cytostatics are not specific enough to target only tumour cells and also affect the healthy cells of exposed individuals (Besse et al., 2012; Deblonde and Hartemann, 2013; Gajski et al., 2016a, 2016b; Kosjek and Heath, 2011). This can result in genotoxic effects in non-tumour cells and lead to genetic alterations in normal cells and/or secondary tumours in case of cancer patients (Gajski et al., 2016a, 2016b; Kopjar et al., 2006; Toolaram et al., 2014).

A large worker population can be exposed to cytostatics and exposure can occur at different moments during the production and use of these drugs. Exposure can happen during manufacture, transport and distribution, and of course, during its use in health care services, either in hospital or home care settings, or even at its final disposal as waste material. Health care workers who prepare (pharmacists and pharmacy technicians) or administer (nurses) cytostatics or whoever works in areas where these drugs are used may be exposed to these agents through inhalation, dermal absorption or less probably by ingestion (hand-mouth exposure route). The latter two routes of exposure can

* Corresponding author.
** Correspondence to: Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Av. D. João II, Lote 4.69.01, Parque das Nações, 1990-096 Lisbon, Portugal.
E-mail addresses: ggajski@imi.hr (G. Gajski), carina.ladeira@estesl.ipl.pt (C. Ladeira), mgeric@imi.hr (M. Gerić), vgaraj@imi.hr (V. Garaj-Vrhovac), susana.viegas@estesl.ipl.pt (S. Viegas).

http://dx.doi.org/10.1016/j.envres.2017.10.044
Received 5 September 2017; Received in revised form 23 October 2017; Accepted 25 October 2017

Table 1
The cytostatic drugs covered in the present study.

Name	5-fluorouracil	Cyclophosphamide	Paclitaxel
Abbreviation	5-FU	CP	PTX
CAS No.	51-21-8	6055-19-2	33069-62-4
Chemical structure			
ATC code	L01BC02	L01AA01	L01CD01
MW (g/mol)	130.08	279.10	853.91

CAS No., Chemical Abstract Services number; ATC code, anatomical therapeutic chemical classification code; MW, molecular weight.

occur due to contact with contaminated work surfaces, clothing, medical equipment, patient excreta etc. (Kopjar et al., 2006; Mahboob et al., 2012). Other workers, like those employed in the synthesis and production of these products, and the staff involved in the cleaning process, transport from or to the health units, and disposal of hazardous drugs or contaminated material can also be exposed (Kiffmeyer et al., 2012; Meijster et al., 2006). Presently, due to the evolution of the technical protection resources available in the workplaces, such as biological safety cabinets and hoods, the most common and problematic route of exposure is skin absorption rather than inhalation or ingestion, which can be observed even in the most modern healthcare centres (EP (European Parliament), 2016).

Several previously published papers have shown the presence of traces of several cytostatics on pharmacy work surfaces reserved for receiving, storing, preparing, and validating preparations (Fleury-Souverain et al., 2015; Hedmer et al., 2005; Viegas et al., 2014). Contamination was also found in administration areas (Hon et al., 2011; Viegas et al., 2014). Additionally, several studies have found cytostatics in biological samples (blood and urine) from healthcare workers (Fransman et al., 2007a, 2007b; Sessink et al., 1994; Sottani et al., 2008). Workers are normally exposed to lower doses than cancer patients but this can still promote adverse health effects (Moretti et al., 2015). Indeed, there have been many papers reporting health effects among workers that handle cytostatics (Turci et al., 2011; Yoshida et al., 2011; Zhang et al., 2016a; Zhang et al., 2016b). Some of these encompass reproductive toxicity that can result in spontaneous abortions, temporary or permanent infertility, preterm births, congenital malformations, and learning disabilities in offspring (Connor et al., 2014; Fransman et al., 2007b; Stover and Achutan, 2011). Spontaneous abortions have been reported approximately twice more often among exposed pregnancies than unexposed ones (Stücker et al., 1990); the same goes for congenital malformations, infertility, and possibly leukaemia, as well as other cancers (Froneberg, 2006; Kopjar et al., 2009; Stücker et al., 1990).

Moreover, long-term occupational exposure has been linked with increased risk of skin rashes, hair loss, irritation, hypersensitivity, and headaches after reported skin contact (Chu et al., 2012; Hedmer et al., 2008; Stücker et al., 1990), infections attributed to a decrease in white blood cell count and immunological suppression, organ toxicity (e.g., liver, kidney, lung, and cardiac toxicity), myelotoxicity, mucosal ulcers, fatigue, bleeding, and headaches (Fransman et al., 2007a). Furthermore, the incidence rates of DNA damage, chromosomal abnormalities, and cancer occurrence increased among occupationally exposed individuals, consistent with the inherent carcinogenic potential of several of these drugs (McDiarmid et al., 2010; Rombaldi et al., 2008; Yoshida et al., 2006).

An important aspect that should also be considered is the fact that cytostatics are often used in combination with two or more drugs to achieve synergistic effects on tumour cells resulting from their differing MoA. However, most if not all of these chemical agents are generally nonselective, and along with tumour cells, normal cells may undertake cyto/genotoxic damage (Kopjar et al., 2006; Villarini et al., 2012). *In vivo* exposure to cytostatics has been shown to induce different types of lesions in DNA, depending on the particular stage of the cell cycle at the time of the treatment. Although the toxicological profile and MoA of each individual drug is well characterized, the toxicological interactions between drugs are likely but poorly established in an occupational exposure context (Ladeira et al., 2016). Additionally, the multiple contamination found on the workplace surfaces reported in a previously published work (Viegas et al., 2017, 2014) supports the idea that workers are probably exposed to a mixture of cytostatics emphasizing the need for additional toxicological screening of their mixtures to gain a better understanding to what extent occupational exposure to the mixture can result in higher risk for workers' health (Cavallo et al., 2005; Gajski et al., 2016a; Kopjar et al., 2006).

Previous research already demonstrated possible additive and/or synergistic action when exposure to more than one drug occurs. In one of the cases, a combination of 5-fluorouracil (5-FU), etoposide (ETO) and imatinib mesylate (IM) was tested showing additive and/or synergistic action and drew attention because of a higher risk than could be assumed from the studies evaluating the effect of a single substance (Gajski et al., 2016a).

The aim of the present study was to evaluate possible cyto/genotoxicity of three cytostatic drugs in a mixture; 5-FU, cyclophosphamide (CP), and paclitaxel (PTX) (Table 1) using human peripheral blood lymphocytes (HPBLs) as an in vitro model. HPBLs are commonly used in chemicals testing (OECD, 2014) as reference cells for a large number of assays and are proven to be good surrogate cells. They were selected as a suitable test system since they are widely applied in genetic and environmental toxicology both in vitro and in vivo and represent an appropriate and readily available source of primary cells with a stable genome. They are obtained in a relatively non-invasive way, do not require special pre-treatment, and perform well in the comet as well as cytokinesis-block micronucleus (CBMN) assay (Collins, 2004; Fenech, 2000; Natarajan and Obe, 1980). Specific cytostatics were selected based on our previous study where we conducted an assessment of workplace surface contamination of pharmacy and administration units in two Portuguese hospitals (Viegas et al., 2014). Besides, specific cytostatics were selected by the fact that they are among the most consumed anticancer drugs and based on their different MoA. For the assessment of DNA strand breaks and genomic instability after treatment with the selected cytostatic drugs mixture, two complementary assays; the comet assay (Azqueta and Collins, 2013) and CBMN assay (Fenech et al., 2011) were used. This study strives to provide new knowledge about the impact of such mixtures on non-target, human circulating blood cells that could facilitate future occupational risk assessments.