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Mutagenicity assessment of environmental contaminations in a hospital centralized reconstitution unit

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ABSTRACT

Introduction: Cytotoxic drug exposure of hospital staff preparing intravenous chemotherapy is a major issue and related mutagenic risks should be more explored. The aim of this study was to assess the mutagenicity of several cytotoxic mixtures prepared at fixed concentrations, and the mutagenicity of environmental samples collected in a hospital centralized reconstitution unit. In parallel cytotoxic exposure in environmental samples was quantified.

Methods: Environmental samples were performed by wiping method using swabs in five critical production unit areas. Mutagenicity was assessed with a liquid microplate AMES test using two salmonella typhimurium strains (TA98 and TA100), in prepared cytotoxic mixtures containing 14 cytotoxic drugs (cyclophosphamide, cytarabine, dacarbazine, docetaxel, doxorubicin, epirubicin, etoposide, 5-fluorouracil, gemcitabine, ifosfamide, irinotecan, methotrexate, paclitaxel and pemetrexed) according a dichotomous strategy and in environmental samples. Cytotoxic drugs were quantified in samples using liquid chromatography coupled to mass tandem spectrometry.

Results: Mutagenesis was observed for the mix of 14 cytotoxic drugs with TA98 strain ± S9 fraction but not TA100 strain. After dichotomous approach, only doxorubicin and epirubicin exposure were associated to mutagenesis. The mutagenesis observed was expressed at lower concentrations with the mix of the 14 drugs than with anthracyclins alone, assuming a synergistic effect. Despite measurable level of cytotoxic contamination in environmental samples, no mutagenesis was highlighted in Ames tests performed on these environmental samples.

Conclusions: The analyses carried out show the conservation of the mutagenicity of cytotoxic drugs found in very low quantities in the environment. The traces of cytotoxic drugs found in our unit regularly exceed the limits given by some authors. This approach may be considered as a new tool to monitor environmental contamination by cytotoxic drugs.

1. Introduction

Injectable chemotherapies are centrally manufactured in hospital pharmacies. The increased need for anticancer preparations and the centralization potentiate the occupational exposure to cytotoxic drugs. Since 1986, safe handling practices guidelines have been defined (OSHA, 1996; International Society of Oncology Pharmacy Practicioners Standards Committee, 2007; Department of Health and Human Services, 2014; Easty et al., 2015; Sessink et al., 2016). Despite these guidelines, occupational exposure to cytotoxic drugs remains a major issue for the protection of hospital staff (pharmacy technicians, pharmacists and nurses). Indeed, antineoplastic drugs are potentially genotoxic (Cavallo et al., 2005; Villarini et al., 2011; Ladeira et al., 2015; Moretti et al., 2015) and may cause cancer (Hansen and Olsen, 1994; International Agency for Research on Cancer, 2000). Although workers handling antineoplastic drugs are well instructed about risks of exposure, detectable levels of these drugs are still reported in their urine and in facilities where drugs are prepared and administered (Falck et al., 1979; Bussières et al., 2012; Maeda et al., 2010; Sabatini et al., 2012; Berruyer et al., 2015). Environmental contamination level

Abbreviations: LC-MS/MS, Liquid Chromatography coupled to mass tandem spectrometry; IS, Internal Standard; LOQ, Limit of Quantification

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may be assessed using surfaces wiping as recommended in the literature (Connor et al., 2016), although no standardized procedure has been validated so far. To date, no cross-sectional studies have been carried out in order to assess in parallel the mutagenicity and the cytotoxic level of exposure of environmental samples.

The objective of this study was to evaluate the mutagenicity of the 14 most manipulated cytotoxic drugs in Paris Saint-Louis Hospital reconstitution unit: cyclophosphamide, cytarabine, dacarbazine, docetaxel, doxorubicin, epirubicin, etoposide, 5-fluorouracil, gemcitabine, ifosfamide, irinotecan, methotrexate, paclitaxel and pemetrexed. According to the IARC classification, only two drugs were considered to be carcinogenic for human: cyclophosphamide, an alkylating agent used in blood, breast, ovarian, lung, head and soft tissue cancers; and etoposide, a topoisomerase inhibitor used in testicular, lung, blood, ovarian and head cancer (American Society of Clinical Oncology, 2018). The 12 other drugs are classified as 2B (dacarbazine), 3 (methotrexate and 5-fluorouracil) or unclassified. These 14 drugs represented 95% of the total annual mass handled and 58% of the preparations carried out (in number) in Paris Saint-Louis Hospital reconstitution unit. More precisely, the objective is to evaluate the mutagenicity of several cytotoxic mixtures prepared at fixed concentrations in order to assess additive or synergistic effect of the cytotoxic mixture and the mutagenicity environmental samples collected in a hospital centralized reconstitution unit. In parallel, the 14 cytotoxic drugs were quantified in environmental samples.

2. Material and method

2.1. Chemicals

Cyclophosphamide monohydrate; cytarabine; dacarbazine; docetaxel; doxorubicin hydrochloride; epirubicin; etoposide; 5-fluorouracile; gemcitabine hydrochloride; ifosfamide; irinotecan hydrochloride; methotrexate hydrate; paclitaxel and pemetrexed disodique and their stable labeled isotopes used as internal standards (IS) were purchased from Alsachim (Illkirch, France). Methanol LC-MS, acetonitrile LC-MS (LC-MS Chromasolv®, 99%), dimethylsulfoxyde (DMSO) (Chromasolv® Plus, for HPLC, ≥ 99,7%) and formic acid (for mass spectrometry, 98%) were purchased from Sigma-Aldrich (St. Louis, USA). The ultra-pure water was obtained by a Milli-Q® system from Merck Millipore laboratory (Darmstadt, Germany). Individual stock solutions of each analyte and IS were prepared at 1 $mg.mL^{-1}$ in DMSO.

2.2. Environmental sample collection method

2.2.1. Wiping method

Sampling method was performed by wiping work surfaces of 225 cm^2 using a dry swab with a viscose head (Copan Diagnostics, Murrieta, USA), wetted with 50 µL of sterile water. Two successive wipes, wet then dry, were performed according to guidelines recommendations (Connor et al., 2016). The viscose head was then immersed in 2 mL of methanol LC-MS grade and samples were vortex mixed for 30 s and placed in ultrasonic bath for 10 min.

2.2.2. Wiping method validation

The wiping method consisted of a wiping step and a desorption step. The desorption step was validated by spiking appropriate dilutions of 14 cytotoxic drugs mixture on dry swabs desorbed according the procedure described above. The wiping step was validated by spiking appropriate dilutions of 14 cytotoxic drugs mixture on metal surfaces wiped according the procedure above, and by wiping the same surface using a third swab that was desorbed then quantified by LC-MS/MS.

2.3. LC-MS/MS method

Cytotoxic were quantified using liquid chromatography coupled tandem mass spectrometry. Quantification of 5-fluorouracil was performed using a Hypercarb™ Porous Carbon column (100 × 2.1 mm, 3 µm) (ThermoScientific, Waltham, USA), a mobile phase mixture gradient of water and methanol, and an electrospray negative ionization detection mode. An Acquity BEH C18™ column (2.1 × 50 mm, 1.7 µm) (Waters, Milford, USA), a mobile phase mixture gradient of water and acetonitrile with formic acid 0.1% were used to quantify the 13 other drugs using electrospray positive ionization mode: cyclophosphamide, cytarabine, dacarbazine, docetaxel, doxorubicin, epirubicin, etoposide, gemcitabine, ifosfamide, irinotecan, methotrexate, paclitaxel and pemetrexed. Calibration ranges were performed after desorption. Calibration standards were set at the following concentrations: 0; 0.5; 1; 5; 10; 50; 100 and 200 $ng.mL^{-1}$, after desorption. The concentration of quality controls was 7.5; 25; 150 $ng.mL^{-1}$. The quantification limits (LOQ) were found to be 0.5 $ng.mL^{-1}$ or 0.6 $pg.cm^{-2}$ for gemcitabine, cyclophosphamide, epirubicin and doxorubicin; 1.0 $ng.mL^{-1}$ or 1.3 $pg.cm^{-2}$ for 5-fluorouracil, dacarbazine, methotrexate, pemetrexed, ifosfamide, irinotecan and paclitaxel; and 5.0 $ng.mL^{-1}$ or 6.3 $pg.cm^{-2}$ for cytarabine, etoposide and docetaxel.

2.4. Ames test

2.4.1. General principle

The Ames test is performed using Ames MPF™ 98–100 (a liquid and miniaturized microplate assay format, similar to a Mini Ames test) purchased from Xenometrix (Allschwil, Switzerland) using two salmonella typhimurium strains: TA98 and TA100. Ames tests were also performed with S9 fraction, derived from rat liver treated with aroclor-1254. The S9 fraction induces the metabolism of xenobiotics and allows the study of drugs requiring metabolic activation. The tests were performed in triplicate. The results were read visually by turning the colored indicator and counting well and were expressed in revertant count. According to the Xenometrix technical sheet (additional file 1), two scores were used to measure mutagenicity: the fold induction over the baseline score and a binomial test to ensure that the prototrophy reversion is specifically due to analyte exposure. Prototrophy reversion was positive with a fold induction over the baseline score greater than or equal to 2 and a "binomial b-value" below 0.01. Ames tests were conducted on prepared cytotoxic mixture samples and on environmental samples.

2.4.2. Exposure strategy

Ames test exposures were conducted using 10 µL of prepared cytotoxic mixtures or environmental samples extracts.

2.4.2.1. Prepared cytotoxic mixture. The dichotomous strategy presented in Table 1 was set up in order to limit the number of tests conducted. A semi-logarithmic concentration range was used to provide a wide exposure range. In mixtures, each level of the concentration range shown in Table 1 displayed the same concentration for every cytotoxic drug. The first test was performed on the 14 cytotoxic drugs mixture. Tests No.2, No.3 and No.4 were defined according annual preparation frequency: 82.5%, 10.1% and 3.3%, respectively. Depending on the results of tests No.2, No.3 and No.4, the drugs causing mutagenesis were pooled by mechanism of action in test No.5. A final test was performed (test No.6) with the 3 most frequently used drugs (5-fluorouracil, cytarabine and cyclophosphamide) at therapeutic concentrations: 100; 500 and 1000 $mg.L^{-1}$. Concentration ranges were chosen according previous published occupational studies (Schierl et al., 2009; Merger et al., 2014; Fleury-Souverain et al., 2015).

2.4.2.2. Environmental samples. Environmental samples were collected 3 consecutive days in 5 critical areas chosen in the hospital centralized reconstitution unit of Pharmacy Unit in Saint Louis Hospital: raw materials storage area, handling area, release zone area, manufactured preparations storage area and control laboratory area. Day 1, collected