Handling of cytostatic drugs and urine mutagenesis

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Summary. As part of a French national epidemiologic study on human reproduction among hospital personnel, we investigated urinary mutagenicity of nurses and personnel from oncology units exposed to cytostatic drugs. During a first series of experiments, urine mutagenicity of 47 subjects working in six oncology units was investigated in the Marseille regional's hospital. A control group of 37 individuals working in one cardiology clinic was also included. Urinary mutagens were extracted on XAD-2 resin and tested by two bacterial mutagenicity tests: the Ames test with tester strains *Salmonella typhimurium* TA 97, TA 98, TA 100 and TA 102 with or without metabolic activation (S9 MIX) and the SOS Chromotest with tester strain *Escherichia coli* PQ 37-S9 MIX. Bactericidal activity towards the tester strains was found in 40% of the urine samples (36/90). During a second series of experiments, urine mutagenicity of 17 office clerks was also investigated. Toxicity was found in six of the 21 urine samples. No significant difference of toxicity distribution and no relationship between toxicity and cigarette smoking were found. Qualitative analysis of the data showed no significant difference among the exposed groups and the control group (Chi 2 = 0.529, df = 2) with tester strain TA 98 + S9 MIX. Cigarette smoking was found to be the main factor of increased urinary mutagenicity (Chi 2 = 0.529, df = 1). Quantitative analysis of the data showed that mutagenic potencies varied from 0.332 ± 0.539 revertants/mg creatinine to 7.226 ± 6.743 revertants/mg creatinine with TA 98 + S9 MIX. A relationship between the number of cigarettes smoked and mutagenic potency was found (Spearman rank coefficient r = 0.412, P < 0.05). One urine sample was found to be mutagenic with tester strain TA 102 and PQ 37.

Key words: Cytostatic drugs – Mutagenesis – Hospital nurses – Ames test – SOS Chromotest

Introduction

Many cytostatic drugs have been shown to be mutagenic, carcinogenic and teratogenic in laboratory animals (Sorsa et al. 1985). Recent studies have suggested the possibility of an occupational health hazard among hospital personnel who handle these compounds. Increased urinary mutagenicity has been found in nurses (Falck et al. 1979; Bos et al. 1982; Anderson et al. 1982; N. Guyen et al. 1982) and in pharmacists (Anderson et al. 1982; Kolmodin-Hedman et al. 1983). Biological monitoring with mutagenicity assays has also produced negative results (Gibson et al. 1983; Venitt et al. 1984). Two bacterial assays have been used to evaluate urinary mutagens: the Ames test with *Salmonella typhimurium* strains TA 1535 and TA 100 (Ames et al. 1975) and the *Escherichia coli* WPR2 uvrA technique (Green and Muriel 1976).

During a French epidemiologic study conducted by the Institut National de la Santé et de la Recherche Médicale (INSERM) on human reproduction among hospital personnel, we took the opportunity to monitor exposure of cytostatic drugs to nurses and other personnel working in oncology units. Biological monitoring was performed with the Ames test using tester strains *S. typhimurium* TA 97, TA 98, TA 100 and TA 102 and with the SOS Chromotest using tester strain *E. coli* PQ 37.
Materials and methods

Chemicals

Amberlite XAD-2 resin (60–150 mesh) was purchased from Serva (Heidelberg, FRG). Aroclor 1254 was from Analabs (North Heaven, CO, USA). Glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide phosphate, sodium salts (NADP), dimethyl sulfoxide (DMSO), o-nitrophenyl-β-o-galactopyranoside (ONPG) and the 5-bromo-4-chloro-3-indoly-β-galactoside (X-Gal) were from Sigma (St. Louis, MO, USA). Acetone was analytical grade.

Subjects

All subjects were women under 45 years of age and belonged to the Centre Hospitalier Régional de Marseille (France) which includes 12535 employees. Individuals taking vitamin C or antibiotics or salicylates during the three preceding weeks were excluded. The “exposed” subjects were selected among six oncology units chosen because of the monthly doses of chemotherapy. Group 1 included 29 individuals who handled the cytostatic drugs. These individuals prepared 12 to 15 intravenous admixture solutions/week. Each subject in this group was exposed to an average of six cytostatic drugs (3–11) at high dosimetry. The most frequently handled drugs included cyclophosphamide, methotrexate, vincristine, adriamycin, cisplatinum, etoposide, 5 fluorouracile and bleomycin. The individuals used various protective measures to handle the cytostatic drugs: no safety protocol (n = 5), gloves (n = 4), gloves + masks (n = 2), gloves + masks + vertical laminar flow hoods (n = 18). Group 2 included 18 subjects in contact with the drugs or their metabolites through the biological fluids of treated patients, but not administrating therapeutics.

During the first series of experiments, 35 individuals who worked in one cardiology unit were selected for the control group (Group 3). An additional control group of 17 office clerks (Group 4) was included in the second series of experiments and determination of urine mutagenic activity was repeated for 12 subjects from the different groups. This work involved 99 subjects and 111 urine samples were analyzed.

Collection of urine samples and concentration of urinary mutagens

Urine samples (150 ml) were collected after three working days as recommended by Falck et al. (1979) and the samples were stored at −25°C until assayed during a blind study.

The extraction and concentration of mutagens were carried out by the method of Yamasaki and Ames (1977). Following thawing, the urine samples were filtered through Whatman paper no. 1 and mutagens were extracted on Amberlite XAD-2 columns (4 cm²/100 ml of urine). The columns were washed with 10 ml of Milli-Q water and the mutagens were eluted with 10 ml of acetone. The eluate was evaporated to dryness under a stream of nitrogen. The organic residue was dissolved in DMSO (400 µl/100 ml urine) and stored at −25°C. For each batch of extractions (9 columns), a control was included by replacing the urine sample by Milli-Q water.

Determination of creatinine levels in urine samples

Creatinine levels in urine samples were determined by the Jaffé reaction using the automated technique of Chasson et al. (1961).

Bacterial mutagenicity assays

Two bacterial mutagenicity assays were used during this work: the Ames test and the SOS Chromotest.

Ames test. Samples of concentrated urine samples were assayed for mutagenicity with the Ames test (Maron and Ames 1983) using S. typhimurium tester strains TA 97, TA 98, TA 100 and TA 102. These bacterial strains were kindly provided by B. N. Ames. This set of strains is auxotrophic for histidine and revert to prototrophy by frameshift mutations (TA 97 and TA 98) and by base-pair mutations (TA 100). The new strain TA 102 can detect a variety of mutagens which are not detected or detected poorly by the other strains. In the plate incorporation assay, three doses of urine concentrates were tested: 40, 60 and 80 µl, corresponding to 10, 15 and 20 ml of urine samples respectively. After a 48-h incubation period, the number of spontaneous and observed revertants were counted on each plate and the number of induced revertants was calculated (observed revertants–spontaneous revertants) for each tested dose. The dose-response curve was calculated by regression analysis from the linear portion of the curve. The slope of the curve was defined as the mutagenic activity of the urine sample (expressed as revertants/ml urine). Mutagenic potency was calculated as the mutagenic activity/mg creatinine (expressed as revertants/mg creatinine). For the epidemiologic study, several results were considered:

(a) mutagenic urine sample (+). A mutagenic urine sample was considered with the following parameters: (i) doubling of the spontaneous frequency of revertants and (ii) dose-response relationship.

(b) mutagenic and toxic urine sample (+T). A mutagenic and toxic urine sample required: (i) doubling of the spontaneous frequency of revertants, (ii) dose-response relationship and (iii) decrease of the number of observed revertants at higher doses.

(c) toxic urine sample (T). A toxic (bactericidal) urine sample requires a 50% decrease at least in the spontaneous frequency of revertants.

(d) negative urine sample (−). A negative response was the last possibility: (i) no doubling of the spontaneous frequency of revertants, (ii) no dose-response relationship and (iii) no toxic effect.

Due to limited amounts of concentrated urine sample, a screening technique of the Ames test was also used: the Spot test. One single dose (10 µl) was applied on a plate which was seeded with the tester strain.

SOS Chromotest. Concentrated urine samples were also tested by the SOS Chromotest using tester strain E. coli PQ 37 (Quillardet and Hofnung 1985). E. coli PQ 37 was generously provided by M. Hofnung. In this tester strain, the lacZ gene (gene for β-galactosidase) is under the control of the SfiA gene (gene for the cell division inhibition), which is one the inducible SOS functions. The induction of the SOS repair system (error-prone system) can be easily measured by the determination of β-galactosidase concentrations in the cells. This measure can be performed by a simple colorimetric assay with X-Gal as the substrate in the spot test and ONPG in the standard test. Moreover, toxicity of urine samples can be estimated by assaying a constitutive gene PhoC (gene for the alkaline phosphatase) by a colorimetric technique.

In the standard test, the enzyme activities were expressed in units (Quillardet and Hofnung 1985) and the ratio of β-galactosidase levels to alkaline phosphatase levels were determined for each tested dose. The induction factor was calcu-